Introduction

Dengue viruses (DENVs) are the most prevalent arthropod-borne viruses worldwide, and are endemic in more than 100 countries throughout Africa, America, and Asia [1, 2]. It is estimated that anywhere from 50-100 million individuals are infected by a DENV each year, ranging from an asymptomatic infection to a life-threatening haemorrhagic fever. Despite the fact that the number of dengue cases worldwide has been steadily increasing since the 1980’s, there are no approved treatments or vaccines [2-4]. The World Health Organization aims to reduce dengue morbidity by 25% and mortality by 50% by 2020 [5]. However, there are currently no approved vaccines, as the presence of 4 antigenically distinct serotypes, along with the concept of antibody-dependent enhancement, has posed a major challenge to development.

Dengue Virus Characterization

DENVs are enveloped, positive-stranded RNA viruses belonging to the Flaviviridae family, and are transmitted through the Aedes aegypti mosquito [1, 6, 7]. The DENV genome is 10.7 kb, and includes a 5’ methyl guanosine cap, a 5’ untranslated region (UTR), and a 3’ UTR [3]. The genome is translated as a single polyprotein which is cleaved into 3 structural proteins [capsid (C), premembrane (prM), and envelope (E)] and 7 nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) [3, 7]. DENV enters the cell through receptor-mediated endocytosis and, as with all flaviviruses, replication and translation occurs in the cytoplasm [3, 8]. The E protein is the primary determinant of DENV serotypes, and acts as the major antigenic target of the host response, while the prM protein acts as a chaperon for the E protein during assembly, and is believed to aid in maintaining the structural and antigenic integrity of the E protein [3, 9]. There are four antigenically distinct DENV serotypes, known as DENV-1, DENV-2, DENV-3, and DENV-4, which are characterized by ≥30% difference in their amino acid sequence, specifically in the E protein [1, 2, 6]. There are also multiple genotypes within each serotype, which are characterized by >6% difference within the nucleotide sequence of the E protein [2]. DENV-1 is composed of 5 genotypes, DENV-2 is composed of 6 genotypes, while DENV-3 and DENV-4 are both composed of 4 genotypes [2].

There are several reasons for this genetic diversity. First, as with all RNA viruses, DENVs contain an error-prone RNA-dependent RNA polymerase, which is thought to result in one mutation per round of replication [2]. Second, homologous recombination is thought to play a role in diversity within the viral population, as co-infection with multiple genetically diverse
genotypes has been shown to be possible, both within the mosquito vectors and human hosts, resulting in recombination of DENVs [2]. However, there are no reports of recombination between serotypes [2]. Lastly, the increase in population size, density, and migration of both the mosquito vectors and the human population has increased the transmission rate of DENVs, and resulted in the introduction of new serotypes and genotypes into naïve populations [2]. Moreover, the increase of both commercial and military transport over the past century, along with ecological and demographic changes in tropical zones, has resulted in the expansion of the habitat of the A. aegypti mosquito [3, 6]. Additionally, the increase in population size and density is thought to further facilitate DENV transmission [6]. Today it is estimated that 3.9 million people live in dengue-endemic areas [8].

Dengue Infection

DENV is injected into the skin of the human host via the saliva of a female A. aegypti mosquito [8]. Symptoms generally manifest for 5 days, following an initial incubation period of 4-7 days [8]. It is estimated that DENVs infect 500 million individuals each year, with ~20% of these infections, or 100 million, being symptomatic [8]. These symptomatic infections can range from a mild infection, known as dengue fever (DF), to a more severe illness, known as dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS) [2, 6]. DF is a self-limiting illness which manifests as an acute fever coupled with a frontal headache or retroorbital pain, along with myalgia, arthralgia, vomiting, and weakness [3, 6]. A maculopapular rash often appears 1-2 days after fever abatement, and the majority of patients begin to recover without complication around 10 days after the initial onset of illness [6]. However, ~2% of symptomatic dengue cases result in DHF/DSS [3]. The clinical signs of DHF/DSS initially mirror those of DF; however, a dramatic decline in platelets, vascular leakage, and often haemorrhage is seen at the moment of defervescence [2, 3, 6, 8]. Symptoms can also include abdominal pain, persistent vomiting, and hypotension [6]. In cases of DSS, severe bleeding, multi-organ involvement, and hypovolaemic shock is seen [8]. It is postulated that 20,000 individuals succumb to a DENV each year [3]. It is unknown why some DENV infections progress to DHF/DSS; it is thought to be a mixture of risk factors, such as gender, the genetic characteristics of the infected individual, immunological status, epidemiological factors, and viral strain [6]. However, the most important risk factor for DHF/DSS seems to be secondary infection with a heterologous DENV serotype, which has been shown to result in a phenomenon known as antibody-dependent enhancement (ADE) [2, 6]. In
general, flavivirus neutralization requires binding by more than one antibody [7]. During a primary DENV infection, antibodies are raised against the E and prM proteins, and neutralization occurs when the number of antibodies bound to a virion exceeds a certain threshold [7]. ADE occurs when the non-cross-neutralizing antibodies which were raised against a primary dengue infection bind to the heterologous, secondary infecting virus, forming an infectious immune complex [7]. These infectious immune complexes are then taken into Fc-receptor bearing host cells [7]. While the exact mechanisms of ADE have not yet been elucidated, it is thought that infection of Fc-receptor bearing host cells by these immune complexes results in an increased number of infectious cells and an increased viral output [7].

It is important to note that not every secondary dengue infection results in DHF/DSS, but that it is simply a prominent risk factor. Additionally, DHF/DSS is often observed in infants <1 year old, despite these children never having been previously infected by a DENV [7, 10]. It has been suggested that while passively acquired maternal antibodies are meant to protect against a DENV infection, after a certain time period, these antibodies actually enhance a primary dengue infection, as the body treats it as a secondary dengue infection [7]. It is also important to note that the severity of DHF/DSS increases proportionally to the time between infections, and severe dengue has been observed up to 20 years following primary infection [6, 7].

Evidence for Antibody-Dependent Enhancement

A unique opportunity to study the effects of severe disease and ADE in relation to secondary infection presented in the country of Cuba, as the majority of the population shares similar genetic factors, it’s medical and research infrastructure is well-integrated, and several epidemics caused by a single serotype have occurred in the country [6].

A seroepidemiological survey carried out in 1975 revealed that only 2.6% of the Cuban population had DENV antibodies. Almost all of these individuals were 45 years old or older, indicating that DENV had not been present in Cuba since at least the end of World War II [6]. That is until 1977, when an epidemic was caused by the introduction of DENV-1 [4, 6].

Serological evidence estimated that up to 44.5% of all Cubans were infected with the DENV-1 strain throughout the course of the 1977 epidemic [6]. Importantly, no cases of DHF/DSS were reported throughout this time period [6]. Following this initial DENV epidemic, there were three epidemics in Cuba resulting in DHF/DSS cases: DENV-2 was introduced to Cuba in 1981, causing the first DHF/DSS epidemic [4]. A second DHF/DSS epidemic, caused by DENV-2,
began in 1997, followed by DENV-3 in 2001 [6]. Interestingly, there was a higher risk of DHF/DSS in individuals secondarily infected with DENV-2 in 1997 versus those infected with DENV-2 in 1981, supporting the hypothesis that the severity of a secondary infection is directly proportional to the time between infections [6].

**Vaccine Development**

DENV vaccine development has proven difficult for a multitude of reasons. The immune functions necessary for sufficient protection against DENV are currently unknown, there are currently no sufficient animal models, and it must be cost-effective in order to be accessible in many of the resource-poor countries where dengue is endemic [3, 9]. Most importantly, a vaccine must result in immunity against all 4 DENV serotypes simultaneously, in order to avoid eliciting a nonneutralizing antibody response which would result in ADE upon infection with DENV [3, 9]. The four DENV serotypes are sufficiently antigenically different that it is considered necessary to produce four monovalent vaccines, which can then be combined to form a tetravalent vaccine which protects against infection with all four serotypes [6]. However, this may result in an issue known as viral interference, which occurs when one DENV serotype replicates at a higher efficiency than others, resulting in a dominant immune response [3]. A number of promising viral vaccines have been developed in recent years, including inactivated, live attenuated, recombinant subunit, viral vectored, and DNA vaccines [3, 9]. Each potential vaccine focuses on eliciting a neutralizing antibody response, and many of these vaccines have progressed to Phase II and III trials [3, 9].

The most prominent vaccine developed against DENVs is ChimeriVax, a live recombinant tetravalent vaccine [3, 5]. In the case of ChimeriVax, the E and prM structural genes of DENVs were cloned into the 17D strain of yellow fever, a live, attenuated yellow fever vaccine strain [3]. The yellow fever 17D vaccine is the most successful flavivirus vaccine to date, due to both its rapid onset and longevity of immunity [3, 9]. Importantly, pre-existing immunity thanks to prior yellow fever vaccination does not affect the induction of a neutralizing antibody response to DENV [3]. ChimeriVax was first licensed in Mexico in 2015, and has since been licensed in 20 countries worldwide [5]. It is administered in 3 doses, at 0, 6, and 12 months, and is used for individuals between the ages of 9 and 45 [5]. As ChimeriVax is a live vaccine, viral interference has been observed, with vaccine efficiency being higher in serotypes 3 and 4 than in serotypes 1 and 2. Additionally, the vaccine has been shown to perform differently in seropositive and
seronegative individuals, with an increased risk of DHF/DSS in seronegative individuals beginning approximately 30 months after the first dose [5]. Despite this, the benefits of ChimeriVax remain positive, and the WHO simply recommends that countries interested in licensing ChimeriVax should adopt a pre-vaccination screening strategy, in which only seropositive individuals are vaccinated [5].

There are two additional vaccine candidates currently in Phase III trials [5]. The first has been developed by NIH/Butantan, and involves using site-directed mutagenesis to delete 30 nucleotides in the 3’ NTR, resulting in attenuation [3, 5, 9]. Deletions in the 3’NTR allow for the induction of both T-cell and antibody responses against both structural and nonstructural DENVs protein, which has been deemed an important advantage against DENV infection [3]. The second vaccine candidate was developed by Takeda, and is a live attenuated recombinant chimeric tetravalent vaccine [11]. The E and prM genes of DENV-1, DENV-3, and DENV-4 are expressed in an attenuated DENV-2 backbone, resulting in a robust neutralizing antibody response, and cellular immune response [11].

**Vector Control**

In the absence of an approved therapeutic, alternative approaches such as vector control measures have proved beneficial in terms of DENV control. Trials in Vietnam involved encouraging communities to place a natural predator of *A. aegypti*, the crustacean *Mesocyclops*, in areas where the mosquitoes are known to congregate [6]. Over the course of several years, a reduction in both *A. aegypti* mosquitoes, and in the incidence of DENV infection, was observed [6]. However, this approach has been criticized as it is likely only effective in communities with a certain attitude concerning Dengue [6]. A secondary alternative approach involves infecting *A. aegypti* mosquitoes with the bacterium Wolbachia, which has been shown to induce resistance in mosquitoes to a host of arboviruses [6, 12]. Additionally, Wolbachia reduces the adult lifespan of mosquitoes by 50% and is transmitted transovarially to offspring [6, 12]. Trials using Wolbachia are currently underway [12].

**Conclusion**

While Dengue continues to pose a serious threat to global health, the development of efficient vaccines is dire. While the existence of four antigenically distinct serotypes, combined with the phenomenon of ADE has proved incredibly difficult to overcome, the development of
ChimeriVax, along with the vaccines currently undergoing Phase III trials, show promise for the future of Dengue.