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Lessons Learned and Recent Advances in Dengue Research

Juan Samuel Sulca Herencia

Abstract

Dengue is the most important arbovirus, many research have contributed to the diagnosis, management, prevention and control of this disease, which will be described in this chapter, for example: the importance of serotypes and genotypes for the development of the disease, the relationship of the viral load between symptomatic and asymptomatic people, the influence of antibodies on the development of the disease, co-infections with microorganisms and chronic diseases, possible reservoirs, the diagnostic assays, cross-reactions in the diagnosis, the influence of climate change on the disease and the vector, mechanisms of transmission of the disease, new drugs and plant extracts with antiviral activity, the dengue vaccine, the results of immunizations, etc. This information gives a concrete idea of the advances and challenges against this disease.

Keywords: dengue, dengue virus, arbovirus, flavivirus, *Flaviviridae*

1. Introduction

Dengue is a single systematic and dynamic disease that includes severe and non-severe clinical manifestations [1]. It is caused by any of the four dengue virus serotypes and it is transmitted by *Aedes* mosquito bites, being the main vector *Aedes aegypti* [1]. Dengue can be maintained in an urban cycle which involves humans and it is a serious health problem worldwide [1]. For the past decades, this disease has been spread alarmingly due to different factors like climate change, migration of people, tourism, lack of access to basic services, etc. [1, 2]. Not only does it affect a large portion of the world's population but also it offers lessons in health sector, research and epidemiology which must be skilled to help in better understanding of the cycle of this disease, set out control strategies and lead the way to future investigations. Thus, in this chapter we are going to discuss some of the main lessons learnt throughout work experiences with this disease and we will learn new strategies designed for studies, assessment and control.

2. Dissemination of the disease in urban areas and transmission mechanisms

Dengue is the most important and common arbovirus in more than 100 countries [1]. This disease is caused by one of the four serotypes of dengue virus (DENV), more than one dengue serotype can be found in many geographical areas (phenomenon called hyperendemicity) [1]. Outbreaks of this disease have been

reported in America, Africa, Mid-west, Asia and the Pacific islands [2]. Nearly 3 billion people (40% of world population) are at risk in areas where dengue occurs, and about 70% of the population at risk are in South East Asia and Western Pacific region [2]. About 400 million people are infected with dengue annually, of which 100 million people are sick and 22,000 die of severe dengue [3]. A virus, a vector and a sensitive population must be in the same geographic area for a dengue onset outbreak [1]. Travelers contribute to dengue dissemination to non-endemic areas but they may also serve as sentinels for warning dissemination. Some studies indicate that dengue represents about 2% of the diseases from travelers returning to from endemic areas [4]. Based on GeoSentinel, a data-collection network between 1997 and 2006, dengue was imported from South-east Asia (51%), South Central Asia (17%), Latin America (15%), the Caribbean (9%), parts of Africa (5%) and Oceania (2%) [4]. The number of febrile travelers returning from the tropics and sub-tropical areas being diagnosed with dengue has increased from 2% in the 1990s to 16% by 2005 [4]. A study of paediatric travelers in 19 countries reported dengue and typhoid fever as the most often febrile diseases in children returning from tropical regions and sub-Saharan Africa [4]. *Aedes aegypti* vector can be disseminated relatively easy in urban areas because of its strong anthropophilic habits, its biological features like egg resistance to desiccation, its permanence within and not away from the urban centers, and the search for water with low load of organic matter for its oviposition making its arrival in rural areas, where the temperature enables its replication, relatively simple; transform cemeteries, tire repair shop, bus terminals, etc. in critical points of infestation where the vector surveillance must be carried out [5]. The virus can reach new areas where cases of symptomatic and asymptomatic persons or cases of people's incubation period of the disease have not been reported. Around three-quarters of dengue infections presented each year are clinically inapparent, the asymptomatic ones were considered dead-end hosts because they do not produce high enough viremia to infect mosquitoes [6]. Some studies provide evidence that despite a low level of viremia, dengue asymptomatic persons were capable of transmitting the disease to mosquitoes and potentially enabling a high virus transmission. Since it has no symptoms, they could continue in their day-to-day work and be exposed to mosquito bites [6]. The virus can also be spread through infected mosquitoes so that once the mosquito is infected and the extrinsic incubation period is passed, it can be a lifelong carrier of the virus which is about 2 weeks or a month. The interruption of dengue transmission was possible in the 60s and at the beginning of the 70s as a result of an *Aedes aegypti* eradication. The lack of surveillance and vector control followed outbreaks in the Caribbean, Central America and South America [1]. From this experience, it is considered that vector control and epidemiological services detecting and studying dengue cases have to work together and maintain a constant vigilance [1]. The monitoring service should be capable of making the difference between a seasonal and/or temporary increase and increases in the number of cases resulting from a dengue outbreak so that tools of epidemiology as the endemic channel are highly important. If the number of cases reported is higher in two standard deviations above the endemic channel in monthly or weekly notifications, a warning of dengue is produced [1, 7]. However, the lack of notifications continues to be one of the most significant problems in order to prevent and control this disease in many countries as well as in the western Pacific region [1]. The Geographic Information Systems (GIS) allows the analysis of this information on the geographical reference basis in space and time. This type of systems is important for vector-borne disease surveillance and vector control strategy planning [8]. DENV can be transmitted to humans through the bite of female mosquitoes of the *Aedes aegypti*. Other dengue transmission mechanism

is the perinatal transmission and it occurs when a mother is infected near the childbirth so that the infection can be transmitted through microtransfusion when a placental abruption occurs or during delivery when the mother's blood gets in contact with the newborn's mucosal. Dengue in pregnancy is related to premature delivery, fetal distress in labour, intrauterine death and miscarriages. DENV can also be transmitted through breast milk or exposure to blood, organs or infected tissues like bone marrow [9]. In a study carried out at the Brazilian blood center from February to June in 2012, donors tested positive for DENV-3 and DENV-4 were found and 42 units of PCR tested positive were transfused in 35 recipients. Of these findings, 16 units testing positive were transfused in 16 sensitive recipients in which 5 possible cases transmitted by transfusion (TT) were found, 1 case was considered as a possible TT, and 10 of them were not transmitted. However, no significant differences were found between symptoms and mortality in cases and controls [10].

3. Dengue virus serotypes and genotypes and its relation to clinical cases

The evolution of molecular biology and phylogenetic analysis has enabled molecular epidemiology studies binding DENV-phylogenetic analysis which was found in different geographic areas within a certain period of time and clinical and epidemiological data in order to establish a relation to genotypes and lineages which are found with other circulating strains as well as to identify the origin and the transmission route, the severity of the disease, etc. There are four related DENV serotypes but antigenically different DENV-1, DENV-2, DENV-3, DENV-4 and each of them generates an immune response to the infection. The nucleotide sequences show 63–68% homology within the DENV group in comparison with 44% between DENV and Yellow Fever virus (YFV), and 51% between DENV and West Nile virus (WNV), and it shares a minor homology at 80% at a level of amino acids [11]. Previous studies based on partial (prM/E), partial or complete E gene; or complete genomic sequences recognized distinct DENV genotypes [12] (**Table 1**).

The genotypes may present different lineages or clades. The genetic changes in the virus are caused by mutations or the introduction of a new variant from other region, thus genotyping of strain virus is used to identify the epidemic outbreak source and spread [12]. Some dengue genotypes are related to a higher virulence. DENV-2 and DENV-3 Asian genotypes are associated with severe infections [1]. In South East Asia, dengue hemorrhagic fever (DHF) and/or dengue shock syndrome (DSS) outbreaks are caused by DENV-2 southeast Asian genotype strain, meanwhile epidemics caused by DENV-2 in Latin America presents solely dengue fever (DF) in most cases. The DENV-2 genotypes circulating in the United States seem to be less virulent. Because of the introduction of the South East Asian genotype virulent, the number of cases of DHF and DSS in America has increased [1, 11].

It is thought that an infection with DENV serotype provides lifelong immunity against the same serotype infection and short-term protection against infection by a 2 or 3 month-heterologous serotype [1, 11]. Some studies contradict the hypothesis that has been accepted until now. In 2010–2011 a study in DENV-2 American/Asian genotype outbreak was conducted in Iquitos, Peru. It was carried out 15 years after the first DENV-2 American genotype in that region. The results on the study showed that protection against homologous DENV-2 may be incomplete [13]. In another study carried out in Peru, it was found that the antibodies of a primary infection against DENV-1 neutralized more efficiently in American DENV-2 than in Asian DENV-2. It is believed that this cross-protective immunity is not so strong to inhibit viremia but it may contribute to reduce DENV-2 infection

Dengue serotype	Genotype	Geographical distribution
DENV-1	I	Southeast Asia, China, The Middle East
	II	Thailand
	III (Sylvatic)	Malaysia
	IV	Countries of the Pacific Rim, the Western Pacific, islands and Australia
	V	The Americas, West Africa and Asia
DENV-2	Asian I	Thailand, Malaysia, Cambodia, Myanmar, Vietnam and Australia
	Asian II	China, Indonesia, The Philippines, Taiwan, Sri Lanka, India, Honduras and Mexico
	Southeast Asian/ American	Southeast Asia, Central and South America and the Caribbean
	Cosmopolitan	East and West Africa, the Middle East, the Indian subcontinent, Indian and Pacific Ocean Islands, Australia, Mexico
	American	Central and South America, the Caribbean and the Indian subcontinent and the Pacific Islands
	Sylvatic	West Africa and Southeast Asia
DENV-3	I	Southeast Asia, the Philippines and the South Pacific islands
	II	Continental Southeast Asia
	III	Asia, East Africa, the Americas
	IV	Puerto Rico and Tahiti
DENV-4	I	The Philippines, Thailand, Vietnam, Myanmar, Malaysia, Sri Lanka, India
	II	Southeast Asia (Indonesia, Malaysia, Singapore), China, islands of the Western Pacific Ocean, Australia, the Caribbean and the Americas
	III	Thailand
	IV	Malaysia

Table 1.
Dengue virus serotypes and genotypes and its geographical distribution.

symptomatology [14]. In 2013 evidences of a new dengue serotype appearance (DENV-5) that was identified in serum samples collected in 2007 during an epidemic in Malaysia were documented. Initially, it was thought that DENV-4 cases will circulate between primates and *Aedes nivalis* mosquitoes in the woods at South East Asia. However, it was shown that this virus was genetically different to DENV-4 from the rainforest and it had certain similarities to DENV-2 when the virus was isolated and after sequencing the whole genome, *rhesus macaques* were infected with four dengue serotypes. Once recovered from the infection, the monkeys were inoculated with DENV-5 which produced different antibodies. Moreover, it was observed that the infection with DENV-5 virus titer was four times higher than other serotypes. On the basis of this, it was concluded that DENV-5 will be a new serotype. It is thought that this new serotype may mainly circulate in non-human primates (NHP). This new serotype has only been found in the Woods of Sarawak [15]. However, there is a possibility that this new serotype may spread to human population becoming a public health concern. Thus, phylogenetics and epidemiological surveillance studies are required.

4. Factors that increase dengue severity, cases and co-infection classification

Most of dengue infections are subclinical or asymptomatic. Dengue epidemics were presented in Cuba in 1981 and 1987, and most cases of dengue shock and hemorrhagic dengue were mainly found in white population than in afro-descendants [16]. In studies carried out in Brazil and El Salvador, it was found that the African descent was a protective factor in dengue hemorrhagic manifestations [16]. In a dengue outbreak in Santiago de Cuba in 1997, it was found that hemorrhagic dengue cases were reported more frequently in patients aged between 15 and 39 years old. Additionally, they found a history of asthma in a 16.5% of the cases [17]. The WHO estimates that, by 2030, the diabetes mellitus will have been the 7th leading cause of death. A study carried out for evaluating the influence of diabetes mellitus and its relation to clinical manifestations of dengue, indicated higher risks of dengue symptoms potentially fatal within patients developing diabetes mellitus [18]. Other risk factors such as sickle-cell disease, uremia, allergies, hypertension, chronic renal failure may enable disease severity [1]. Regarding age, the lower compensation capacity of capillary plasma extravasation in children increases dengue shock risk. It has been observed that serious dengue cases were continuously presented in primary infections from breastfeeding babies whose mothers were developing immunity to some dengue serotype. The non-neutralizing antibodies produced by cross reaction during a primary infection or passively obtained from a mother to newborns are adhered to epitopes of dengue virus infections facilitating the entry of cells to Fc-receptors. This may contribute to a person's viral load increase resulting in strong immune response that includes inflammatory mediators [19]. Cytosines may enable plasma extravasation. In secondary infections, memory T-cells caused by previous reactions to dengue activate, replicate, produce cytosines and die of apoptosis. This can be correlated with disease severity [16]. The patient's background is important when ruling out other causes of this disease, for instance, other flavivirus presence like YFV, Saint Louis encephalitis virus (SLEV), Zika virus (ZIKV), WNV. Alphavirus like Chikungunya (CHIKV), Mayaro virus (MAYV), Venezuelan Equine Encephalitis virus (VEE), Bunyavirus like Oropouche virus (OROV), group C virus, Guaroa virus (GROV), Influenza virus, arenavirus, filovirus as well as other microorganisms can cause the disease with symptoms with similar symptoms such as malaria, Leptospirosis, typhoid fever, rickettsia diseases, etc. [1]. Dengue infections with different serotypes as well as the sequence of these infected patients may influence on the severity of the disease. In a study carried out in Singapore, between 2005 and 2011, febrile adult patients found in DENV-1 cases that were associated with dengue hemorrhagic fever (adjusted RR = 1.74) and severe dengue (adjusted RR = 2.1) were assessed, while DENV-2 had a low risk of dengue hemorrhagic fever (adjusted RR = 0.5) [20]. A special attention should be given to the clinical features of this disease in order to learn differences that lead to the identification of microorganisms causing the disease. It was found that there were no elevated hematocrit or shock cases in CHIKV infection as compared to dengue cases with high hematocrit in 40–69% of cases and shock in 10–39% of cases when comparing the clinical data and laboratory features within dengue infections and chikungunya [21]. The arthralgias were more frequent in CHIKV in 70–100% of cases as compared to at least 10% of dengue cases. The Zika infections may present Oedema in limbs as compared to dengue and chikungunya which is low or nil. The presence of this type of differences does not exclude atypical complications and presentations of the disease, thus the laboratory diagnosis plays a crucial role [22].

DENV, ZIKV and CHIKV are transmitted by *Aedes aegypti* vector. Therefore, the areas where the disease is spread may be the same leading potentially to

co-infections. Co-infection cases within different dengue serotypes have been reported in various countries like Peru where isolation of DENV-1 and DENV-3 in the department of Madre de Dios was reported [23]. Although this type of findings, the connection between dengue co-infections and severity of the infection needs to be further examined. Reports of DENV and CHIKV co-infections have been exhibited since 1964 in Asia, Africa, the Caribbean, North America, South America. Dengue and malaria co-infections have been reported in various countries such as India, Pakistan, Indonesia, Japan, Malaysia, Brazil and many others; and even co-infections among dengue, malaria and chikungunya have been reported in South Africa and Asia. A systematic review to determine global prevalence and distribution of malaria, dengue and chikungunya coinfections reported evidence of coinfections within these agents in 42 countries. The most prevalent coinfection was malaria/dengue followed by dengue/chikungunya, Malaria/Chikungunya and Malaria/Dengue/Chikungunya [24]. Clinical presentations between dengue and malaria are alike so it can sometimes cause a co-infection misdiagnosis. Anemia is a major symptom of infections caused by malaria which are not presented in dengue cases but it is often in this type of co-infections. On the other hand, lowering of platelets and hemoglobin content, reduced aspartate aminotransferase levels and elevated alanine aminotransferase levels are also observed [25].

In 1970, dengue disease was classified as non-classical dengue fever, classical dengue fever, dengue hemorrhagic fever and dengue shock syndrome. Sometime after this, various studies reported lack of correlation between classification and disease severity [26]. This classification showed a high specificity in the identification of hemorrhagic dengue; however, its sensitivity was low when detecting severe dengue cases requiring medical care and/or hospitalization. Thus, the WHO proposed a new classification according to the level of clinical severity by dividing dengue disease in two categories, severe dengue and non-severe dengue in 2008 [1]. This classification makes possible to establish the management and improve notifications for a better epidemiological surveillance so that warning signs and early warning of severe cases requiring hospitalization are proposed [1]. Regarding a study conducted to compare both classifications, it was found that the sensitivity of the new classification to detect severe cases was 65%, and the old classification was 30%. The new classification had 72% of sensitivity to detect patients needing advanced medical services and the old classification only had 32%. Further studies alike indicate that the WHO classification in 2009 has brought benefits in epidemiology and clinical use, some others have proposed to include other variables on severe dengue categories in order to increase sensitivity in a case detection that may require advanced medical care [27].

5. Dengue vectors and mechanisms of infection

Dengue virus is mainly transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes. *Aedes aegypti* is a tropical mosquito regarded as the most important disease transmission vector in urban areas, there are also other vector viruses like ZIKV and CHIKV. Dengue outbreaks have also been attributed to *Aedes albopictus*, *Aedes polynesiensis* and various species of complex *Aedes scutellaris* [1]. *Aedes aegypti* is a species with a strong predilection for human blood adapted to urban zones, especially in human dwellings; it is an efficient vector due to certain features like the egg-laying in a wide range of containers, the egg resistance to drying and the capacity of the female mosquito to bite in multiple occasions until they meet their need of blood. *Aedes albopictus* has adapted to temperate and tropical climates and it is a zoophilic and anthrophilic species and as well as *Aedes aegypti*, they both feed

during the day [1]. Some laboratory studies have reported that *Aedes albopictus* can become infected and transmit other 22 arboviruses such as CHIKV, Eastern equine encephalitis virus (EEEV), Ross river virus (RRV), Western equine Encephalitis (WEE), YFV among others [28]. The vertical virus transmission (transovarial or transovum) of an infected female mosquito to its eggs and subsequent progeny provides a mechanism as the arbovirus may be maintained in nature during climate conditions such as cold periods in temperate and hot regions, and dry in tropical areas or during the lack of susceptible vertebrate host. Many flaviviruses like YFV, Japanese encephalitis virus (JEV), SLEV, WNV can be transmitted in a vertical manner in its natural vectors [29]. Such natural transmission for DENV has already been approved by *Aedes aegypti* and *Aedes albopictus*; however, it occurs in a smaller proportion in *Aedes aegypti*. Moreover, its potential for transmission and maintenance of disease in nature has not been established yet. The preference of *Aedes aegypti* for little organic matter in water or clean water when it comes to lay eggs provides information about the possible places that these mosquito larvae may be found. However, some studies reported that septic tanks have been identified as places where a high *Aedes aegypti* replication can occur [30]. A study in Brazil assessed rudimentary cesspits as possible *Aedes aegypti* breeding sites identifying immature and adult forms of *Aedes aegypti* in cesspits. The average number of insects found was similar to the one reported in common breeding habitats which confirmed the new place of breeding of this vector [30]. Thus, the identification of new *Aedes aegypti* breeding sites indicates a change in habits or an adaptation of the mosquito in its environment that also need to be considered in vector control strategies of *Aedes aegypti*. Climatic factors play a significant role in its biological cycle and displacement of dengue vector. In a study about the effect of temperature on mosquitoes, it was found that mosquitoes maintained at 20°C were less sensitive to the infection and died in less time which lessened the likelihood of midgut infection without affecting the extrinsic incubation period of DENV as compared to a constant 10°C temperature range [31].

6. Dengue virus jungle cycle and reverse zoonosis

DENV can circulate in jungle cycles where virus can remain in place using *non-human primates* (NHP) as reservoirs or viruses transmitted in an urban cycle can infect non-human primates ('spillback' or 'reverse zoonosis') [12]. The urban and jungle cycles are shown to exist in Asia and Africa, in countries such as Malaysia, Senegal, the Philippines where DENV would be identified by a virus isolation from species such as *Presbytis obscura*, *Erythrocebus patas*, and *Macaca fascicularis* respectively [32]. Studies conducted in South East Asia reported antibodies in non-human primates in Indonesia, the Philippines, Cambodia, Vietnam and Malaysia. DENV viral isolation or identification by RT-PCR in NHP has not been reported in Americas but wild caught patas monkeys (*Erythrocebus patas*) and rhesus macaques (*Macaca mulatta*) showing antibodies against DENV by PRNT were reported in a study carried out between 2010 and 2012 in Puerto Rico [32]. As no evidences of DENV jungle cycle in America were shown, it is believed that these results may represent a spillback infection and non-human primates would get the infection of urban cycles presented in the population. In Argentina 2020, antibodies against DENV-1 y DENV-3 in howler monkeys (*Alouatta caraya*) were found; and in Brazil between 2006 and 2014, low antibodies against DENV in free-living golden headed lion tamarins (*Leontopithecus chrysomelas*) were found [32]. These cases were also assumed to result from spillback infection. A study in Thailand in 2008–2009 identified 6 DENV positive-dogs by RT-PCR and/or viral isolation in urban areas,

2 were DENV-2 positive and 4 were DENV-3 positive in a rubber plantation area [33]. It is necessary to continue with this type of studies in these animals in order to develop a viremia high enough to infect mosquitoes. More than 200 viruses from 27 families, including *flaviviridae*, was isolated or detected in bats and several studies have shown DENV nucleic acids and/or antibodies present in Neotropical wildlife. A study in Costa Rica suggest that bats are infected accidentally by DENV because the RNA quantification in blood was low below the minimum infectious dose of mosquito that is needed to maintain the transmission cycle for the virus. Thus, they were considered as dead-end hosts for dengue virus [34].

7. Problems of dengue diagnosis

A series of multiple variant testing have been carried out for dengue diagnosis depending on a person’s infection phase, these tests are conducted with different immunological targets for virus recognition, a part of its structure or a reaction in an infected person or an animal’s body indicating an exposure to DENV [35] (Table 2).

In a dengue study, diagnostic tests display numerous problems which we will describe briefly. The presence of false positive as a result of contamination and different variants of RT-PCR such as endpoint PCR, nested PCR, real-time PCR as well as the use of different primers and enzymes lead to variations in levels of sensitivity and specificity. Some commercial test enables the identification of different microorganisms by utilizing RT-PCR, PCR tests and the detection of the product in about an hour. The RUO Film Array Global Fever Panel tests are utilized for the detection of 6 bacterias, 4 protozoans and 9 viruses like Chikungunya virus, Crimean-Congo hemorrhagic fever virus, Ebola virus, Lassa virus, Marburg virus, West Nile virus, Yellow fever virus, Zika virus and Dengue virus. Whole blood (EDTA) is used as a sample. The use of automation equipment can help to reduce contamination problems and deliver results in a short time as well as providing diagnosis for 19 pathogens. Regarding to improvements for this type of systems the equipment can only process one sample at a time, and the study is expensive. The rapid tests do not need sophisticated equipment or qualified staff, it is feasible in situ and they are inexpensive. Nonetheless, its sensitivity and specificity are not as higher as other techniques. Cell cultures are often used for dengue virus isolation. Many cell lines are used for virus isolation; however, their sensitivity can vary depending on the type of cell line, the clone, the system used for viral isolation and the sample type used for isolation [35, 36]. In order to isolate DENV, it is preferable to use C6/36 cell line obtained from *Aedes albopictus* mosquito salivary glands that is replicated at 28°C as

Diagnostic test	Immunological target
Polymerase chain reaction (PCR)	RNA detection
Rapid tests	NS1, IgM, IgG
Virus isolation	Virus
Immunofluorescence (IF)	Virus, IgM, IgG
Plaque assay (PA) and fluorescent focus assay (FFA)	Virus (titer)
Enzyme-linked immunosorbent assay (ELISAS)	NS1, IgM, IgG, IgA
Neutralization tests	Neutralizing antibodies (IgG)

Table 2.
Most commonly used diagnostic tests for diagnosing dengue.

it has higher sensitivity than Vero or BHK-21 (mammalian lines). Some researchers suggest using a variety of C6/36 HT which grows at 33°C. Such cell is considered to be more sensitive than the traditional C6/36, and it is recommended to use the shell vial or shell vial modified method instead of the traditional or standard isolated system in order to achieve better results. In the modified shell vial method, the cell culture is infected, centrifuged and incubated for 10–15 days, then positive cultures by indirect immunofluorescence are identified [35, 36]. Many laboratories do two or three passages from cell cultures following an assessment of virus presence conducted by IFI. This increases more the number of isolations but the cost of the testing goes up and the time needed to obtain the results raises. The quantification or viral load may be run by real time PCR utilizing a standard reference that in comparison with the sample allow us to assess the number of genetic material copies from the sample [35, 36]. When it is intended to quantify infecting viruses in biological samples for producing antigens or vaccines, is preferred to use PA in Vero or BHK-21 cells [35, 36]. One alternative for viral titration, depending on the study performed and especially when evaluating viral load in mosquitoes, is the use of FFA that is a mixture of plaque assay and immunofluorescence test and it allows the use of C6/36 cells containing higher sensitivity than the above-mentioned cells [35, 36]. IF and ELISAS tests are relatively low cost and they may occur commercially or may be designed. Many of their components as antibodies and antigens can be produced in the laboratory at lower costs but it is necessary a good quality control of production so that there can be variations in job titles of lots that may affect test results [35, 36]. Neutralization tests can be performed under different techniques such as PRNT, microneutralization, microneutralization-ELISA. These tests facilitate the high specificity type IgG neutralizing antibodies detection. However, the levels of sensitivity in relation to the cell line, the strain of virus and the technique used are due to cell cultures. Other problems have yet been overcome are DENV cross-reactions with other flavivirus and within the same serotypes in serological tests like ELISAS, IFI and even considered to be gold standard like neutralization test by plaque reduction (PRNT) exhibiting cross-reactions. Cross-reactivity between serotypes is frequent and it occurs particularly in epitope on NS protein for the conservation or in conserved epitopes on E glycoprotein and may vary according to homology between every DENV serotype and genotype. The use of specific monoclonal antibodies for every dengue serotype in the IFI tests improve greatly the specificity of the test as compared to the use of HMAF. The use of tests allows to detect multiple pathogens at the same time like CDC DENV-1-4 rRT-PCR multiplex detecting infecting dengue virus serotype and it was approved by FDA in 2013 [35, 37]. The CDC Trioplex rRT-PCR assay uses real-time (TaqMan®) RT-PCR assays for detection and differentiation of RNA from DENV, CHIKV and ZIKV in serum samples, whole blood (EDTA), cerebrospinal fluid. This also enables the RNA detection of ZIKV in urine and amniotic fluids. FDA has authorized the use of this test under an Emergency Use Authorization (EUA) [38].

8. Animal models for dengue

It is not yet found a suitable animal model expressing all characteristics for dengue disease [39]. Among the most common models used for research are mice showing drawbacks with low-level virus replication of clinical samples, and non-human primates showing a problem for not expressing the clinical disease in the same manner as humans [39]. The use of nursing mice used in intracranial inoculations with high viral load inducing neurological diseases and paralysis are still been used; however, its use continues more for the production of biological

supplies. DENV can slightly replicate in mice A/J, BALB/c and C57BL/6. Mice A/J and BALB/c can die of paralysis [39, 40]. For animal studies, it is considered that an infection with DENV 10^4 – 10^6 PFU (plaque-forming units) concentrations imitate inoculum concentrations of a mosquito bite [39]. The mouse model is generally used initially to assess vaccines being the most suitable the immunocompetent mice but when it exhibits low viremia may result in the underestimation of the results. The use of nursing mice is a good way to assess possible candidate vaccines inducing neurovirulence. Although the NHP do not develop the disease, the antibody seroconversion produced is human-like [39, 40].

9. Antivirals for treating dengue

At the moment, there is no antiviral therapy for DENV treatment. It is believed that the compounds working as inhibitors of RNA-dependent RNA polymerase have shown low efficiency. Moreover, there is a possibility that viral epitopes of DENV may trigger an immune cell response, preceded by development of severe disease. Thus, these epitopes are examined as targets for antiviral productions and they are known as DENV entry inhibitors and can be used in combination with inhibitors of the virus replication in order to increase efficiency.

Inhibition of DENV attachment and entry into the host cell can inhibit immune activation.

Various compounds as peptide entry inhibitors, DN59 and 10AN1 may inhibit the antibody-dependent enhancement (ADE) in vitro. The doxorubicin antibiotic SA-17 is structured similarly to tetracycline and it has an antiviral activity against DENV serotype 1, 2 and 3 in Vero and C6/36 cells interfering with viral entry by binding to the hydrophobic pocket of the E-protein without exhibiting virucidal activity. The glycosidase inhibitors are unpopular due to its toxicity and low specificity but may help understand E-protein glycosylation processes. The binding agents to carbohydrates occur only during the stage of virus adsorption to the host cell. Concanavalin and agglutinins of wheat germ can bind N-acetylglucosamine residues and the percentage of DENV-lytic plaques in BHK cells can be reduced. Plant lectins such as *Hippeastrum hybrid*, *Galanthus nivalis*, and *Urtica dioica* inhibit DENV-2 infection in Raji/DC-SIGN cells. The compounds of heparan sulfate are potential recipients for DENV. Dengue E protein domain III is responsible for the interaction of heparan sulfate, and it is believed to be in all DENV serotypes and to have epitopes that are recognized by neutralizing antibodies. The suramin is similar to heparan sulfate and persulfated glycosaminoglycan, they bind to a polyanion-binding site of DENV E protein by inhibiting infection. The sulfated polysaccharides extracted from red algae, carrageenans and DL-galactan presented antiviral activity against 4 serotypes of dengue; however, the antiviral activity of DENV-4 DENV-1 weakened in Vero cells and in human Hepatocytes [41, 42].

10. Dengue vaccine

In May 2019, the U.S. Food and Drug Administration (FDA) approved the use of Dengvaxia[®], a vaccine against DENV that may be used in people aged from 9 to 45 years old [43]. This vaccine utilizes a live attenuated chimeric yellow fever/dengue virus based on a Yellow fever 17D vaccine virus backbone chimerized with prM and E proteins from DENV1–4 replacing the YF prM and E, and 3 doses are administered every 6 months. In phase III trials the overall protective efficacy was 56.7% and 60.8% in South East Asia and Latin America countries, respectively. In 2017, the

vaccine manufacturer, Sanofi Pasteur, announced that some people getting the vaccine without having been previously infected by DENV may be at risk for developing severe dengue if the disease is acquired after immunization [43]. The vaccine candidates utilize strategies such as vaccines of Live-attenuated virus, Inactivated virus, Recombinant protein, DNA vaccine, Viral vector vaccine, Virus like particles and others. Two vaccine candidates (DENVax and TV003/TV005) are being tested in efficacy trials in both Asia and Latin America. DENVax is a tetravalent recombinant live-attenuated dengue vaccine licensed to Takeda. The live virus vaccine utilizes chimerization with DENV-2 PDK-53 as the backbone with DENV-2/-1, -2/-3, and -2/-4 chimeras are created by replacing the DENV-2 prM and E genes with the respective genes from the other DENV serotypes. Other vaccine candidate is TV003/TV00 with a whole attenuated virus for three of four serotypes (attenuated by deletion of 30 nucleotides from 3' UTR of DENV-1, DENV-3, DENV-4, and a chimeric DENV-2/DENV-4) [44]. In spite of having already a vaccine available to prevent dengue, there is a need to further research in order to improve the vaccine for dengue or to produce a new vaccine which enables improvements in certain aspects, for example, a single dose that may immunize for extended periods of time even lifelong, the vaccine effectiveness no longer relying on previous exposure to flavivirus or DENV, the age of group to provide protection being the broadest possible including children under 9 years old, and its administration decreasing the chances of getting the most aggressive dengue disease at no point post vaccination.

11. Conclusions

Dengue is still a major public health concern worldwide. Several natural and social factors have contributed to the number of cases increased in recent decades. The efforts attained in the search of new antivirals and vaccines, and prevention and control strategies for this disease have not proved sufficient. However, the science and knowledge development acquired up to now provide us the tools we need to, and mark the way to follow, in order to achieve a control of this disease.

Conflict of interest

The authors declare no conflict of interest.

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Author details

Juan Samuel Sulca Herencia
U.S. Naval Medical Research Unit No. 6, Universidad Nacional Mayor de San
Marcos, Lima, Peru

*Address all correspondence to: juan.sulca4@unmsm.edu.pe;
juan.s.sulca.ln@mail.mil

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