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Dengue Fever in a One Health Perspective

Latest Research and Recent Advances

Edited by Márcia Aparecida Sperança

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Infectious Diseases

Volume 22

Aims and Scope of the Series

This series will provide a comprehensive overview of recent research trends in various Infectious Diseases (as per the most recent Baltimore classification). Topics will include general overviews of infections, immunopathology, diagnosis, treatment, epidemiology, etiology, and current clinical recommendations for managing infectious diseases. Ongoing issues, recent advances, and future diagnostic approaches and therapeutic strategies will also be discussed. This book series will focus on various aspects and properties of infectious diseases whose deep understanding is essential for safeguarding the human race from losing resources and economies due to pathogens.

Meet the Series Editor

Dr. Rodriguez-Morales is an expert in tropical and emerging diseases, particularly zoonotic and vector-borne diseases (notably arboviral diseases), and more recently COVID-19 and Monkeypox. He is the president of the Publications and Research Committee of the Pan-American Infectious Diseases Association (API), as well as the president of the Colombian Association of Infectious Diseases (ACIN). He is a member of the Committee on Tropical Medicine, Zoonoses, and Travel Medicine of ACIN. Dr. Rodriguez-Morales is a vice-president of the Latin American Society for Travel Medicine (SLAMVI) and a member of the Council of the International Society for Infectious Diseases (ISID). Since 2014, he has been recognized as a senior researcher at the Ministry of Science of Colombia. He is a professor at the Faculty of Medicine of the Fundacion Universitaria Autonoma de las Americas, in Pereira, Risaralda, Colombia, and a professor, Master in Clinical Epidemiology and Biostatistics, at Universidad Científica del Sur, Lima, Peru. He is also a non-resident adjunct faculty member at the Gilbert and Rose-Marie Chagoury School of Medicine, Lebanese American University, Beirut, Lebanon, and an external professor, Master in Research on Tropical Medicine and International Health, at Universitat de Barcelona, Spain. Additionally, an invited professor, Master in Biomedicine, at Universidad Internacional SEK, Quito, Ecuador, and a visiting professor, Master Program of Epidemiology, at Diponegoro University, Indonesia. In 2021 he was awarded the "Raul Isturiz Award" Medal of the API and, the same year, the "Jose Felix Patiño" Asclepius Staff Medal of the Colombian Medical College due to his scientific contributions to the topic of COVID-19 during the pandemic. He is currently the Editor in Chief of the journal Travel Medicine and Infectious Diseases. His Scopus H index is 55 (Google Scholar H index 77) with a total of 725 publications indexed in Scopus.

Meet the Volume Editor

Márcia Aparecida Sperança is an associate professor at the Center for Natural and Human Sciences at the Federal University of ABC (UFABC), Brazil. She earned her Ph.D. in Biology of Parasite-Host Interaction from the Parasitology Department at the Biomedical Institute of the University of São Paulo, Brazil. Dr. Sperança then worked as an assistant professor and researcher at Marilia Medical School, Brazil. Her scientific expertise lies in the diagnosis, molecular biology, and epidemiology of arboviruses, the bacteria Helicobacter pylori, malaria parasites, Chagas disease, leishmaniasis, and COVID-19. Since 2009, Dr. Sperança has been the head of the Laboratory of Pathogenic Agents at UFABC.

Contents

Preface

Dengue fever (DF) is an arthropod-borne disease transmitted by different species of *Aedes* mosquitoes that live in tropical and subtropical regions of the world to non-human primates (sylvatic form) and humans (human form). The etiological agent of DF is dengue virus (DENV), a flavivirus species, grouped into five known distinct serotypes: DENV1, DENV-2, DENV-3, DENV-4, and DENV-5 associated with a sylvatic form. DF control is challenging to human health institutional organizations since the DENV life cycle is complex with the inclusion of arthropod vectors, humans, and animals.

DF can be asymptomatic or exhibit classic symptoms such as headache, fever, nausea, and petechiae. DF can even cause severe haemorrhagic syndrome that can lead to death. The pathophysiology of DF is associated with the host immune system, making it difficult to develop an effective and safe vaccine. Thus, treatment of DF is based on alleviating symptoms. The principal strategy to control DF is through *Aedes* elimination. Environmental changes, including climate and humidity, are important factors to disseminate *Aedes* mosquitoes. Vector biology knowledge can help to find new strategies for *Aedes* control. Improvement of DF diagnosis in vectors and human hosts can help to identify the silencing circulation of DENV and can help to prevent DF outbreaks. Investigation of DF pathophysiology is also important for developing new targets for drug development. Thus, this book discusses DF from a One Health perspective.

The book is organized into five sections: “Epidemiological Aspects”, “Environmental Aspects”, “Pathogenicity”, “Diagnosis and Treatment” and “Management Strategies”.

Section 1 discusses the history of DF prevalence and management in a Chinese county; the modification of DF distribution in American populations with greater prevalence in children younger than 15 years; the re-emergence of sylvatic DENV-2 in Southern Senegal, revealing the role of DF sylvatic form in maintaining virus sources during a long period of time; studies on the silent transmission of DENV by asymptomatic individuals; and the importance of asymptomatic DF in blood safety. Section 2 examines variations in the influence of climate parameters on DF vector biology and the risk of DENV adaptation to sylvatic *Aedes* species. Section 3 describes encephalitis in DF and discusses its biological mechanism. Section 4 outlines diagnosis and treatment strategies with three chapters on the description of primers and probes to detect families of the principal arboviruses by RT-qPCR in clinical samples in a single reaction; the use of chest radiography and an abdominal ultrasound to identify severe DF cases; and employment of a gene-silencing technique to investigate biological aspects and treat DF.

Finally, Section 5 includes chapters on management strategies to impair DF, including a description of a network formation to analyze DF complexity; the use of the Google

Earth-Pro tool to map the distribution of DENV cases; viral genomic surveillance to enable early intervention in DF epidemics; DF reduction through vector control; and the application of a hospital disaster management model used during the SARS-CoV-2 pandemic to severe cases of DF occurring during an epidemic.

I am grateful to all authors for their important contributions and trust, and to IntechOpen for once more allowing me to participate as the editor for a book of high scientific level, contributing in a decisive way to serious problems of public health worldwide.

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Section 1

Epidemiological Aspects

Chapter 1

History of Dengue Fever Prevalence and Management in a One Health Perspective in Hainan Island, China

Qingfeng Guan, Archana Upadhyay and Qian Han

Abstract

Dengue fever (DF), a mosquito-borne viral infection common in warm, tropical climates, is an acute infectious disease caused by the Dengue virus (DENV). Geographically, Hainan Island falls in the southern belt of China holding an approximate area of 33,920 km^2. Meteorologically, Hainan is characterized to have a tropical maritime monsoon climate, giving rise to favorable natural conditions for different mosquito species. However, the diversity of mosquitoes and their abundance has undoubtedly put the island at a higher risk of mosquito-borne viral disease outbreaks. In this chapter, we have discussed the prevalence, control, and management of DF in Hainan Island in China along with the different species of mosquitoes responsible for transmitting the virus. In addition, future prospective of some important DF management strategies, related research methods, and integrated control strategies for the effective control and management of DF with One Health perspective has been summarized.

Keywords: dengue fever, dengue virus, Hainan Island, mosquito monitoring and control, one health

1. Introduction

Since the first reported outbreak in 1779 in Jakarta, Indonesia, many such outbreaks have taken place globally in tropical and sub-tropical climates majorly in urban and semi-urban areas having a wide array of weather conditions [1]. In this major outbreak, DENV type 3 was the causative agent, which was believed to be imported from countries in southeast Asia. Since then, several other imported DF cases have led to major and minor outbreaks in provinces like Guangxi, Yunnan, Hainan, Fujian, etc., in China [1]. Two major outbreaks of DENV type 1 and DENV type 3 were recorded, during 2006–2007 and 2012–2015, respectively [2]. From 1978 to 1991, DF outbreaks in China were mainly concentrated and limited to the coastal areas such as Guangdong and Hainan Province [2]. Hainan experienced the highest incidence rate between the years 1978 and 1992. However, fewer cases have been reported since then.

2. History of DF prevalence in Hainan Island, China

Hainan occurs as the southernmost province and the second largest island in China, having Guangdong province across the Qiongzhou Strait to the northern part of China (**Figure 1**). It boasts of a tropical monsoon climate experiencing rainy season during the months of May till October. The overall climatic conditions are suitable for the breeding of *Aedes* mosquito larvae and for the optimal transmission of DF. Hainan province has experienced three DF epidemics in the past. DENV type 3 was the causative agent for the first outbreak caused in 1978, followed by another outbreak in 1985–1988, and a third one, a dengue hemorrhagic fever in 1991, both of which were caused by DENV type 2 [3, 4]. In October 1979, a large number of suspected dengue cases were found in the northern coastal areas of Dan County (Danzhou city), Hainan Island. Later, the disease spread rapidly along the coastline *via* the transportation lines to the neighboring ports. By 1980, a total of 18 counties/cities and 208 towns, mainly falling in the coastal areas around the island, were facing a major outbreak [3]. In this period, 437,469 DF cases occurred in Hainan Island, and the infection rate was found to be 74% [3, 4]. Its long epidemic period and high incidence rate were of great significance in the epidemiological history. However, the incidence rates decreased significantly in 1981 and almost declined in September 1982 [3].

In early September 1985, suspected dengue cases were reported in Ganchong district along the northern coast of Dan County. Yangpu Township in the county became the local epidemic epicenter in mid-October and reached its peak in late October, which led to further spread of the infection. Neighboring townships in the Ganchong area started experiencing the incidence rates leading to a peak in early November. In late October, most of the adjoining areas along the northern coast started reporting patients, which caused several local outbreaks. However, in late November, cases invaded Changjiang, Lingao counties, and Haikou city, and still outbreaks occurred in a few areas of Changjiang. The outbreak hit Dan, Changjiang, Lingao counties, Haikou city, and 25 towns, with 12,449 cases reported in 3 months having an incidence rate of 210.68/100,000 and 28 deaths. The mortality rate of this

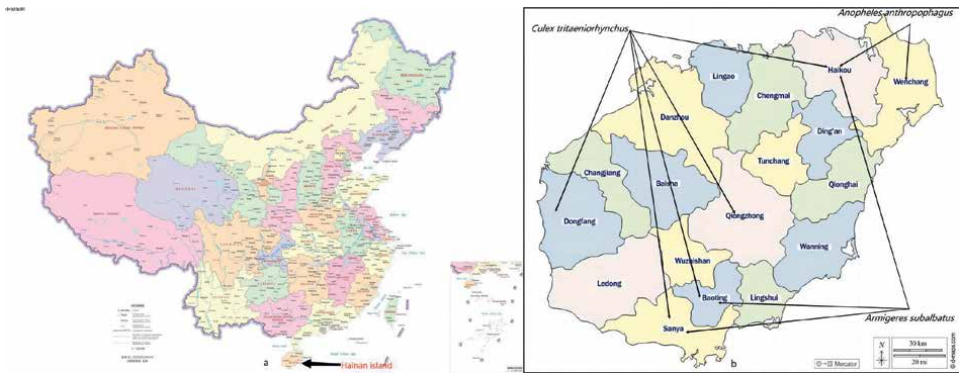

Figure 1.
Geographical representation of the map of Hainan, China, highlighting the prominent cities and counties of the province. A: Map of China, localizing and highlighting Hainan Island. b: Map of Hainan Island displaying different counties and cities harboring different species of mosquitoes due to its typical tropical climate. Three mosquito species were labeled on the map. Map of China was downloaded from the web of Ministry of Natural Resources of the People's republic of China (http://bzdt.ch.mnr.gov.cn/browse.html?picId=%224028b0625501ad13015501ad2bfc0690%22). Map of Hainan Island was downloaded from the web of d-maps (https://d-maps.com/carte.php?num_car=21235&lang=en).

prevalence was 0.47/100,000, and case fatality rate was 2.25 per thousand [5]. In the first half of 1986, the epidemic had gone down owing to the early diagnosis and appropriate control measures of the epidemic. Subsequently, the prevention and control measures failed to persist for a long time, and it gave rise to new waves of epidemic, and finally led to an island-wide pandemic in August and September 1986. In 1986, 113,589 cases were reported, and 289 people died, affecting 182 townships and 27 farms in 18 cities and counties [6]. By 1987, the epidemic had gradually declined, with 30,229 cases and 76 deaths. In 1988, 7379 cases were reported and 18 deaths were reported. By the end of 1988, the outbreak had ended. The 1985–1988 epidemic was due to the DENV type 2 [7].

From June to November 1991, the third epidemic broke out in five cities and counties, with 13 villages and 3 towns falling prey to the epidemic. 521 cases were reported, most of which were mild. 92.3% of the patients were uninfected during the 1985–1988 epidemic, and only 6.8% were reported to have dengue-like symptoms [4, 8, 9].

In 2019, a total of 291 dengue cases were reported in Haikou, with an incidence rate of 12.64/100,000, including 251 local cases (86.3%) and 40 imported cases (13.7%). Among the imported cases, 32 imported cases were reported from Cambodia and Thailand, and 8 imported cases were reported, mainly from Guangxi and Yunnan provinces. All locally confirmed cases were found to be type I, while in the imported cases, all types were reported [10].

3. DENV and its molecular and immunological characterization and identification

Diagnosis of typical cases during epidemics is easy, but the diagnosis depends on virus isolation and serological examination. However, due to lack of awareness, it is easy to miss diagnosis in early epidemics, and other grades of fever may be misdiagnosed as DF. Therefore, DF should be distinguished from influenza, leptospirosis, measles, scarlet fever, and epidemic hemorrhagic fever. In Hainan, malaria needs to be excluded first [11].

DENV is an RNA virus that can be classified in Flavivirus genus of the Flaviviridae family. Being an RNA virus, its genome is undoubtedly prone to mutations, which makes it widespread. DENV has five antigenically different but very closely related serotypes (DENV-type 1, DENV type 2, DENV type 3, DENV type 4, and DENV type 5). The genetic sequences of the DENV1, DENV 2, DENV 3, and DENV 4 are well defined. However, they have some differences in their antigenicity, which makes them graded as reference strains. DENV 1 strain was isolated from Hawaii (DENV-I, Hawaii strain), with DENV 2 from New Guinea (DENV-II, The New Guinea strain), DENV 3 (DENV-III, Philippine H87 strains), and DENV 4 (DENV-IV, Philippines strain H241 strain) from the Philippines. Since then, a large number of DENV strains have been isolated from all over the world. Although some scholars have advocated the classification of some emerging strains with special molecular and immunological characteristics categorizing them into class V serotypes, but the theory has yet to be validated and widely accepted. DF is a mosquito-borne viral infection that gives rise to a sudden onset of fever followed by symptoms such as headache, nausea, muscle and joint pain, and rashes on the skin. It can affect any person but leads to serious complications in immunocompromised people, which can turn out to be fatal. This type of infection can become more fatal and can be named dengue hemorrhagic fever. It can be a life-threatening condition, which may further give rise to the critical form

of infection called dengue shock syndrome. Individuals who have been infected by one DENV serotype can usually have lifelong immunity to the same type of virus but have only partial or temporary protection against the other serotype viruses. Therefore, people living in dengue-endemic areas may develop infections with 4 DENV serotypes. Furthermore, there are common antigen-determination clusters between the 4 DENV serotypes and other members of the flavivirus family, with the presence of cross-reactive antibodies, and hence, the serological identification of different types of DENV becomes complex.

A wide range of laboratory diagnostic procedures have been developed and are in place for confirming DENV infection, which includes the classical method of isolation of the DENV, several molecular-based assays like PCR-based assays for testing the virus, serological assays like antigen or antibodies, or a combination of several assays. DENV can be isolated from the patient specimen or can be detected as the viral nucleic acid or as an antigen, IgM antibody in the blood. A blood specimen with a positive IgM or IgG antibody cannot confirm DENV infection, and can only be clinically diagnosed as suspected or possible cases as acute or convalescent serum samples.

3.1 DENV infection and the body's immune response

The incubation period of the virus ranges between 3 and 14 days, and it can be detected within 4 to 7 days of infection [12]. In many cases, even after collecting the biological specimens during the incubation period, it still at times fails to detect the virus or the corresponding body's immune response. After the onset, the presence of the virus in the blood (viremia period) is about 7 days, and the viral NS1 antigen exists in the blood for a slightly longer time. Within 4 to 5 days after the onset of the disease, the virus can be isolated from the patient's serum, plasma, white blood cells, cerebrospinal fluid, and autopsy tissue specimens, and the detection rate of viral nucleic acid and NS1 antigen is found to be higher during this period. Antibody levels in the patient's blood vary significantly depending on their individual immune status. If the patient has not been previously infected with DENV or other flaviviruses or has received flavivirus vaccine (e. g., Japanese encephalitis, yellow fever, etc.), the first infection slowly increases the levels of specific antibodies, and IgM antibodies appear the earliest, followed by IgA and IgG antibodies. The detection rate of IgM antibodies was about 50% in patients from 3 to 5 days after onset, about 80% in patients from day 5 after onset, and about 99% in patients from day 10 after onset. IgM antibody level reaches the peak 2 weeks after onset, then they gradually decrease followed by which they can be maintained for 2 to 3 months. The IgA antibody usually develops slightly later than the IgM antibody and persists for approximately 45 days [12]. One week after the onset, lower titer of IgG antibody can be detected in the blood specimen, after which the antibody titer persists for several months or even for lifetime. If a patient is reinfected with DENV (previously infected with, or sometimes possibly vaccinated against, or infected with other flavivirus vaccines), antibody titers can rise rapidly and react to a variety of flaviviruses. Mainly high levels of IgG antibodies can be detected in the acute phase of infection and persist for more than 10 months, even for lifetime. IgA antibodies can also be detected in the acute phase specimens. The IgM antibody titers in the early stages of the recovery period are significantly lower than the first infection, or can even be negligible. The application of IgA antibody detection system for detection of the antibodies is still in the evaluation stage.

3.2 Selection of appropriate detection methods

In the early stage of the disease (within 5 days of onset), virus isolation, nucleic acid detection, or antigen detection methods are the most commonly used techniques and methods for diagnosis. When the course of the infection enters the recovery period (after 5 days of onset), serological detection using virus-specific antibodies is generally used for diagnosis.

Virus isolation: Classical isolation of the virus by cell culture methods is the most opted method for the isolation of the virus. It requires a biosafety level (BSL-2) laboratory and related necessary equipment. It is very important to maintain a cold chain during specimen transportation (frozen or refrigerated) for virus isolation. Specimens are usually inoculated in mosquito-derived cells (C6/36) or mammalian cells (BHK21, Vero) for isolation and culture. After the lesions are seen, the virus can be identified by detecting antigens or nucleic acid. Isolation of DENV can be taken as a confirmatory test, however, it takes long time and, therefore, it cannot be suitable for rapid diagnosis.

Nucleic acid testing: A variety of molecular biology-based reverse transcriptional polymerase chain reaction (RT-PCR) methods can be used for DENV nucleic acid detection, including one-step RT-PCR, real-time fluorescent RT-PCR, LAMP (Loop-mediated isothermal amplification assay), RT-RPA (Reverse transcriptase Recombinase Polymerase Activation). Nucleic acid testing identifies viral RNA within 1 ~ 2 days. The detection of viral nucleic acid in patient specimens can be confirmed and subtyped and can be used for early diagnosis. However, it has its own set of drawbacks as it is easy to produce false positives due to number of inhibiting factors, which requires strict zoning operation.

Antigen testing: NS1 antigen detection is commonly done using ELISA method or rapid detection reagent, which can be completed in several minutes to several hours. It is suitable for field and point-of-care settings. It forms an important approach toward acute DF diagnosis, which can be detected within 1 day after the onset, and few other reports have also stated that it can still be detected in blood specimens after 18 days of the onset. Due to the specificity of the NS1 antigen detection method, it can also be used in the differential diagnosis of flavivirus infection.

IgM antibody detection: Capture method ELISA (MAC-ELISA) for IgM antibody detection is the most commonly used detection method, and there are many commercial fast test reagents available for IgM antibody detection, which, however, cannot be used for serotype detection. At present, the detection reagents mainly detect viral envelope protein-specific antibodies, and the major drawback of these tests is that it shows a cross-reaction with other flaviviruses. A positive IgM antibody in the specimen, suggesting that the patient may be newly infected with DENV, is suitable for early diagnosis of DF. However, it is not suitable for single specimen. Even after reinfection, the IgM antibody titer base in blood specimens can still not be detected at times, affecting the diagnostic accuracy for detection of IgM antibodies.

IgG antibody detection: DENV IgG antibodies cross-react with other flaviviruses. IgG antibody test can be used to identify the first; if the acute phase specimen IgG antibody is negative and the recovery phase is positive, it can be determined as the first infection. If the convalescent blood sample is IgG antibody titer than in the acute phase (the two specimens should not be less than 7 days apart). Collecting the second specimen for diagnosis is of great significance for dengue prevention and control, especially in non-endemic areas.

Detection of neutralizing antibodies: The plaque reduction neutralization test (PRNT) and neutralization experiments can be used to detect neutralizing antibodies in the serum, which are the most specific serological tests and have a scope of further typing. However, it requires a contained laboratory infrastructure and is time consuming, therefore, it is not deemed to be suitable for early and quick diagnosis. In this method, the levels of convalescent serum-neutralizing antibodies can be confirmed using this test.

4. Mosquito species and temporospatial distribution in Hainan Island

Hainan province, which is located in the southernmost part of China and is dominated by a tropical Marine monsoon climate, with an annual average temperature of 24.2°C, an average annual rainfall of 1684 mm, and an average relative humidity of 85%. It has the most optimum natural conditions, which are very suitable for mosquito breeding and reproduction. At the same time, under the background of the establishment of the international tourism island and the promotion of the Belt and Road policy, the tourism, trade, and personnel exchanges in Hainan province increase, which gives rise and provides favorable conditions for the infectious diseases mediated by mosquitoes, and further give rise to hidden dangers of disease transmission. Mosquitoes can act as the transmission mode of various viruses and can lead to the epidemics and outbreaks of various mosquito-borne infectious diseases. The mosquitoes in Hainan Province include *Ae. albopictus* (**Figure 2c, f**, & **i**), *Ae. aegypti* (**Figure 2b, e**, & **h**), *Culex tritaeniorhynchus*, *Cx. pipiens pallens*, *Cx. quinquefasciatus* (**Figure 2a, d** & **g**), *Armigeres subalbatus*, *Anopheles dirus*, *An. sinensis*, *An. tessellates*, *An. minimus*, *An. arbumbrosus*, *An. barbirostris*, *An. vagus*, *An. anthropophagus* [14–30] (some distributions were shown in **Figure 1b**).

Ae. albopictus belonging to the genus *Aedes*, is a small and medium-sized black mosquito species and is the vector of DENV and chikungunya virus. *Ae. albopictus* is widely distributed in Hainan Province, mainly in Sanya city [13, 14], Danzhou city [15], Qiongzhong County [14], Lingshui County [14], Lingao County [15], and Baoting County [16, 17].

Ae. aegypti also belonging to the genus *Aedes*, is a dark brown or black medium mosquito species and is an important vector of arboviruses such as Zika virus, DENV, yellow fever virus, and chikungunya virus. It is the dominant mosquito species of DF found in Hainan Province. *Ae. aegypti* is widely distributed in Hainan Province, mainly in Sanya city, Danzhou city, Qiongzhong County, and Lingshui County [18–20].

Although the following mosquitoes do not transmit DENF, we have listed them as a reference for any implication of other vector-borne diseases control. *Cx. tritaeniorhynchus* (**Figure 1b**) belonging to a small brown mosquito species, is an important vector of Japanese encephalitis virus in Hainan Province. They are widely distributed in Haikou city, Sanya city, Dongfang city, Qiongzhong County, and Baoting County and are dominantly found in Haikou city and Dongfang city [21, 22]. *Cx. pipiens pallens* belonging to the genus *Culex*, a hazel small and medium-sized mosquito species is the vector of epidemic Japanese encephalitis virus. It is mainly distributed in northern China and found scantly distributed in Hainan Province [15]. *Cx. quinquefasciatus* belonging to the genus of *Culex*, a medium-sized mosquito species of red brown or light brown, is a vector of various diseases such as Japanese encephalitis in Hainan Province. It is found well distributed in

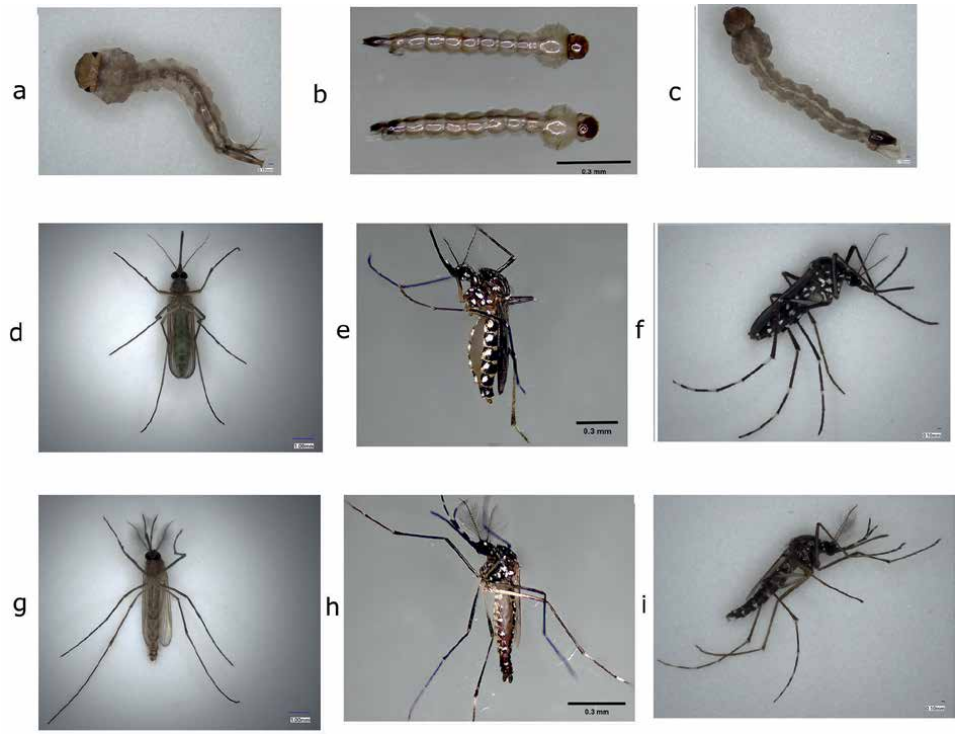

Figure 2.
Morphology of Culex quinquefasciatus, Aedes aegypti, and Aedes albopictus. a, d, and g: fourth instar larva, female adult and male adult of Cx. quinquefasciatus, respectively. b, e and h: fourth instar larva, female adult and male adult of Ae. aegypti, respectively. c, f, and i: fourth instar larva, female adult and male adult of Ae. albopictus, respectively. Photos of Cx. quinquefasciatus and Ae. albopictus were kindly provided by professor Jinbao Gu from the Department of Pathogen Biology, School of Public Health, southern medical university, Guangzhou, China. Photos of ae. Aegypti were provided by Dr. lei Zhang, Laboratory of Tropical Veterinary Medicine and Vector Biology, School of Life Sciences, Hainan University, Haikou, China.

Haikou city, Sanya city, Dongfang city, Qiongzhong County, and Baoting County, and is most dominantly found in Sanya city and Qiongzhong County. However, in the last few years, it has also started appearing dominantly in Haikou city [19]. *Ar. subalbatus* belonging to the subfamily Culicinae, is a large brown-black mosquito species that is the vector of epidemic B encephalitis, which is primarily distributed in Haikou, Sanya, and Baoting County [22]. *An. dirus*, belonging to the genus *Anopheles*, is a gray-brown medium-sized mosquito species that have lesser transmissibility, but can spread other diseases and can endanger health. Hainan province is the main place where *Anopheles* mosquitoes thrive, and are distributed in the areas rich in mountains, jungles, and water systems, such as Wuzhishan city, Qiongzhong County, and Baoting County in the Wuzhishan area, and Dongfang city and Danzhou city along the coastal coast, and hence they are all active areas of *Anopheles* mosquitoes [23–25]. *An. sinensis* is widely distributed in Hainan Province and is widespread in majority of the regions of the province. The areas where these mosquitoes are densely distributed include Haikou city, Sanya city, Changjiang County, Qiongzhong County, and Lingshui County [26–29]. *An. tessellates* belonging to the genus *Anopheles*, are widely distributed in Hainan Province, mainly in Haikou city, Sanya city, Wuzhishan city, Lingshui County, and Lingao County [28]. On the other hand, *An. minimus*, belonging to the genus *Anopheles*, is a tan small

and medium-sized mosquito species. It mainly spreads nonviral diseases, causing serious harm. It is widely distributed in Hainan Province, mainly in Danzhou city, Qionghai city, and Tunchang County [30]. *An. arbumbrosus* belonging to the genus *Anopheles*, is found in Hainan Province, but has a small population, and is mainly found in Wenchang city, Qionghai city, Lingshui County, and Ding'an County [28]. In addition, *An. barbirostris* belonging to the genus *Anopheles*, is widely distributed in Hainan Province, mainly in Dongfang, Wenchang, Qionghai, Lingshui, and Chengmai counties [31]. However, *An. vagus* belongs to the genus *Anopheles* and is less distributed in Hainan Province [28]. *An. anthropophagus*, belonging to the genus *Anopheles*, is a gray-brown medium-sized mosquito species that have not been shown to transmit viral disease. In China, *An. anthropophagus* is distributed in Haikou and Wenchang, Hainan Province [32].

5. Control and management of DF and mosquitoes with One Health perspectives

There is no effective vaccine to date to prevent DF, and most human population is susceptible to the disease. After recovery from infection caused by one serotype, individuals have lifelong immunity to that particular serotype of the virus but lack completely against the other three serotypes. Thus, people living in DF endemic areas may develop infections with DENV type 4 as well in future.

Since 1987, Hainan had spent three years comprehensively controlling the *Ae. aegypti* mosquitos. In 1987, it was in the stage of full implementation planning. Where *Ae. aegypti* mosquitoes exist, measures were carefully implemented according to local environmental and social conditions, and it was required that the Breteau index be controlled below 5 by the end of the year. In 1988, preventive measures and regular management continued to be implemented. By the end of the year, all villages (neighborhood committees, farms) having *Ae. aegypti* mosquitoes had the Breteau index below 5. In 1989, it was the stage of consolidation and validation of the mosquito management. By the end of the year, the Breteau index of *Ae. aegypti* in villages (neighborhood committees, farm companies) was kept below 1. In addition, from 1987 to 1989, two representative villages from each city and county were selected to monitor DF and *Ae. aegypti* mosquitoes annually. The main technical measures in this plan were to adhere to the comprehensive control of mosquitoes in both larval and adult stages, and the specific measures were as follows.

Mosquito larval control: Basic measures include pouring out water in the water tanks, changing the water once every 3 ~ 5 days, adding a lid to some water tanks, and removal of small stagnant water indoors and outdoors. Biological mosquito control includes that water tank was stocked with mosquito fish, *Macropodus opercularis (Syn. M. chinensis)* or *Silurus asotus*, with 1 ~ 2 fish in each tank. Tanks were checked frequently after stocking. For fish that escaped or died, it was necessary to replace them in time. *Bacillus thuringiensis* was placed in water tanks or wells and towers every 7 days.

Adult mosquito Control: Pesticides, such as dichlorvos, fenitrothion, and others, were chosen for spraying so as to kill adult mosquitoes. Villages with a Breteau index of more than 20 (neighborhood committees, farm companies) were subjected to spraying with pesticides twice in February ~ April 1987, each time with an interval of two weeks. The spraying dose was 40 ~ 60 mg of 80% dichlorvos emulsion or

50% chlorvos emulsion per cubic meter room. Doors and windows have to be closed when spraying. During the prevention and control period, once DF occurs, pesticides should be sprayed on the epidemic points or epidemic areas in time to kill poisonous mosquitoes.

In One Health perspective, the health and lifecycle of the zoonotic disease vectors should be explicitly considered alongside the human environment, demographics, and interaction with the zoonotic host vectors. In addition, continuous monitoring from epidemiological point of view has to be taken into consideration [33]. In addition to the increasing range of DENV infection and higher number of infected persons, the increasing frequency of international exchanges, elevation of the urban population, and the lack of effective control measures leading to the deterioration of urban environment and rise of mosquito growth, also needs to be further studied. And these factors along with geographical distribution of DENV make the presence of mosquito transmission vectors even wider.

6. Integrated control and management strategies of DF with One Health perspectives

Certain biological and synthetic control strategies can balance and manage the social, economic, ecological, and health benefits, which has to be carried out in a timely manner and help in combatting the disease in a better manner.

Additionally, carrying out timely and effective vector biological monitoring, practical risk assessment, control, planning, and preparation of vector biological and related diseases, orderly selection of environmentally friendly control technology and comprehensive measures would directly help in eradication. The following six main components of mosquito prevention and control in Hainan Island are as follows:

1. At the time of outbreak, epidemic sites are the core of prevention and control, hence both mosquitoes and their breeding sites have to be in control and managed accordingly.

2. Strengthening and creating awareness among the masses would also contribute to the prevention and elimination of mosquito-borne diseases. Educating the public on the effects of the diseases would create an awareness of the disease.

3. Improving environmental sanitation conditions, removing mosquito breeding sites, and reducing stationary water in the pool and logged water in containers can be useful. Furthermore, rational use of pesticides may be necessary.

4. Establishing and improving the *Aedes* mosquito monitoring network to improve the prevention and control capacity and training the technical personnel at all levels.

5. In addition to the above, special focus on special industries such as flower and bird markets, speeding up the construction of healthy cities, strengthening vector monitoring, and strengthening customs inspection and quarantine measures are a few important strategies to successfully prevent and control the spread of DF.

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Conflict of interest

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Chapter 2

Dengue Fever in Pediatrics

Neydi Osnaya Romero, Sandra M. Villagomez Martinez, Ivan Pilar Martinez and Virginia Diaz Jimenez

Abstract

Dengue continues to be a health problem in the world, according to data from the PAHO. In recent years, dengue cases have been reported from 505,430 cases in the year 2000 to 5.2 million in the year 2019; among the most affected groups are those under 15 years of age. Dengue is a viral disease caused by a virus of the Flaviviridae family, of the Flavivirus genus. It is a disease that requires the bite of the female Aedes aegypti mosquito; the incubation period varies from 8 to 12 days. The pathophysiology of dengue is due to the alterations suffered by the endothelium when caused by the viral particle. Three phases have been identified: 1. the febrile phase; 2. the critical phase, in which patients develop systemic symptoms with a greater inflammatory response, with a risk of bleeding; and 3. the recovery phase. The main symptoms are fever, headache, retro-ocular pain, arthralgia, myalgia, and within the laboratory alterations are elevated hematocrit (hemoconcentration), leukopenia, and thrombocytopenia, among the complications, are pleural and pericardial effusion and ascites, as well like crash and death.

Keywords: dengue, dengue fever, dengue hemorrhagic fever, treatment, children

1. Introduction

Dengue continues to be a severe health problem in the world. According to data from the PAHO, despite the measures to try to contain the number of dengue cases, it continues to be a problem of public health in at least 100 countries. In recent years, dengue cases have increased, probably associated with the increase in urbanization of some areas. This situation conditioned an increase in cases from 505,430 cases in the year 2000 to 5.2 million in the year 2019, and of these 28,000 cases were serious, with a report of 1534 deaths. During the year 2020, of the reported cases of dengue fever, 66% of the deaths correspond to the group of patients under 15 years of age. By the year 2021, 1,324,108 cases of arbovirus were reported, and of these, 89% (1,173,674) of the cases corresponded to dengue fever, the highest incidence of cases is concentrated in the regions of Africa, America, the Eastern Mediterranean, Southeast Asia, and the Western Pacific. During the pandemic, although there was a decrease in infectious diseases, there was also an apparent decrease in dengue cases between 2020 and 2021. This decrease was attributed to an underreporting of cases during the COVID-19 pandemic. However, this decrease in the incidence of infectious diseases did not occur in all countries, since in some countries, such as Pakistan and Thailand, they observed an increase in infectious diseases that were already controlled, such as typhoid fever, measles, and dengue fever, after the confinement [1–4].

Dengue is a viral disease produced by a virus of the Flaviviridae family, of the Flavivirus genus, and there are four serotypes, DENV1, DENV2, DENV3, and DENV4. It is a disease that requires a vector for its transmission. The transmission of dengue fever is carried out by the bite of the female Aedes aegypti mosquito. The incubation period varies from 8 to 12 days, the onset of symptoms is related to the initial viral concentration, cases of dengue hemorrhagic fever have been reported more frequently in patients under 15 years of age, so review this topic as part of the diseases of the pediatric age. In some parts of the world, such as Asian countries, dengue fever has been considered a pediatric health problem. In Latin America, it was considered an entity with a higher incidence in the adult population; however, in recent decades, there has been an increase in the cases in the pediatric population, although not only the cases in the pediatric age have increased, but also the presentation of complications in this age group. Another problem that has been observed in some dengue-endemic cities has been co-infections, con-infections with typhus have been found in India, and during the COVID-19 pandemic, cases of dengue with co-infections with the SARS-COV2 virus were documented in dengue-endemic countries, so in these places, particular interest should be paid to the symptoms of these patients in whom dengue fever is suspected [5–8].

Although it is true that this problem occurs in tropical places and with certain geographical characteristics, we must not forget that it can also be a traveler's disease and that symptoms develop once the patient has returned to their place of origin, so that if a patient presents a fever that is difficult to control, accompanied by headache, joint pain, rash and/or signs of bleeding after traveling to a tropical area, the diagnostic possibility of dengue fever or dengue hemorrhagic fever should be investigated, as the case may be. The cases have been increasing in countries like Brazil, which entails, in addition to being a health problem, an increase in the economic requirements to handle this increase in cases [9, 10].

The diagnosis of dengue fever is established by identifying those tested for the virus from the sixth day by the Enzyme-Linked ImmunoSorbent Assay ELISA technique or by PCR). These tests will be carried out after the fifth or sixth day. Return day for greater diagnostic certainty [9].

2. Clinical manifestations

The pathophysiology of dengue is derived from the alterations suffered by the endothelium when infected by viral particles and the inflammatory response secondary to the infection. During the inflammatory response, the non-structural protein 1 (NS1) of the virus adheres to the vascular endothelium, altering the vascular permeability of molecules and liquids, the coagulation pathways will also suffer alterations, vascular fibrinolysis events will be triggered, and secondarily alteration of platelet adhesion, which generates a problem of thrombosis. These alterations would explain the presence of complications in dengue hemorrhagic fever, the vascular damage will initially cause alterations in the permeability of the endothelium, which, if they persist, can cause lysis of the endothelial cells, once irreversible damage to the endothelium is established. This allows proteins and fluids to leak into the third space, this leak of extravascular fluid will result in hemoconcentration and elevated hematocrit, loss of intravascular fluid that translates as arterial hypotension, which if perpetuated can condition the shock phase, and the presence of complications that lead the patient to death [11–14].

As part of the study of the inflammatory response, some studies have been carried out in which various cytokines have been identified. The type of cytokines that have been identified seem to be related to the infecting serotype in such a way that it has been found that in infections by In DENV2 serotype, the cytokines IL12p70, IL6, and TNF-α are found to be higher than in DENV1 infections; however, interleukin 8 levels are similar in infections by both serotypes. In patients with dengue fever, a greater number of Interferon IF-ϒ than in patients with dengue hemorrhagic fever, the fact that the DENV2 serotype is associated with a higher concentration of cytokines also makes it associated with infections with a greater inflammatory response, and therefore with a greater number of hospitalizations [12].

In a study carried out in Mexico, higher concentrations of IL-12p70, TNF-α, and IL-6 were reported in patients with hemorrhagic dengue fever with the DENV2 serotype than in patients with the DENV1 serotype. However, the levels of TNF-α, IL -12p70, and IL-6 were higher in patients with dengue fever than in patients with dengue hemorrhagic fever infected with the DENV1 serotive. Higher concentrations of interferon (IFN)-γ and IL-12p70 were observed in patients with dengue hemorrhagic fever. If we remember that these cytokines are proinflammatory, it is understood why they are found in higher concentrations in patients with dengue hemorrhagic fever, and why the increase in endothelial permeability results in hemodynamic and coagulation alterations in these patients. Cytokines related to the endothelial inflammatory process, such as IL-12p70, IFN-γ, TNF-α, and IL-6, were higher in patients with dengue hemorrhagic fever. If we remember that these cytokines are pro-inflammatory, it is understood why it is found in older patients. Concentration in patients with dengue hemorrhagic fever and the reason for the increase in endothelial permeability, which allows capillary leakage of fluids, would explain the hemoconcentration, decreased intravascular flow, hypotension, and a greater risk of presenting a state of shock and complications, such as pleural and pericardial effusion and/or ascites [11, 12, 14].

It is evident that the inflammatory response of the patient will depend on the infecting serotype. It must not be forgotten that the different serotypes may be circulating in the same region, it will also influence the response if it is a primary infection or reinfection, either by the same serotype or a different serotype will also affect this response if the patient has other comorbidities, such as arterial hypertension, some immune deficiency or any history that affects the patient's immune response [12, 14].

The clinical symptoms of dengue in pediatrics are variable and sometimes milder than in adults. However, three stages have been described: the febrile stage, the critical stage, and the recovery phase.

In the febrile phase, it occurs between the second and seventh day, there is a fever of up to 40°C, which is mediated by the response of IL1, IL6, and TNF and in this phase, the viremia is recorded by the viral particle that circulates or is associated with lymphocytes, macrophages or platelets. This febrile phase can be accompanied by headache, which is holocranial with retro-ocular pain, myalgia, and arthralgia, predominantly in the long bones, lower back, and lower limbs. A rash that appears between the 3rd and 4th day; some patients show improvement during this phase; patients with a history of a previous infection may present a shorter febrile period and improve or advance to the severe phase [8, 12, 15].

It is in the critical phase that the inflammatory response will cause alterations in the endothelium, which together with hypoalbuminemia will condition the leakage of capillary fluid, favoring the presence of pleural effusion, ascites, and edema in the extremities. At this stage, bleeding data, such as epistaxis, hemorrhages in the skin (petechiae) and mucous membranes, digestive tract can be presented; liver failure

may also occur. It is in this phase that hypovolemic shock, due to hemoconcentration, can occur neurological problems, such as encephalitis, that can be caused by liver failure, should not be ruled out. Dengue shock must be managed in an intensive care area since that if not handled properly can cause the death of the patient. During the follow-up of a patient with a diagnosis of dengue fever, one should be aware of some symptoms that have been described as alarm data that could precede the state of shock, such as abdominal pain, vomiting, drowsiness, and hepatomegaly [13, 14].

During the recovery phase, it is accompanied by the normalization of laboratory abnormalities, such as thrombocytopenia and correction of coagulation times with the consequent reduction in bleeding risks. The recovery phase can appear from 2 to 3 days after the end of the critical phase, also during this phase an itchy maculo-papular rash can be observed, it must be taken into account to make the differential diagnosis with Chinkungunya [10, 16–18].

Laboratory studies should be requested in those patients who observe risk factors, such as blood count, coagulation tests, transaminases, and ammonium levels. In case the patient presents disorders of the state of consciousness, with these studies, we can document hemoconcentration when finding elevated hematocrit, which would be an indication for the use of intravenous crystalloids, decreased platelet count (thrombo-cytopenia), if the patient has active bleeding or is at risk of bleeding, transfusions of platelet concentrates should be performed. With the determination of the coagulation times, if an alteration occurs, the administration of fresh frozen plasma or vitamin K can be indicated as required. In patients with altered state of consciousness and elevated liver transaminases, serum ammonium concentration should be determined, if elevated, antimony measures should be installed. Imaging studies such as a chest X-ray can help us document the presence of a pleural or pericardial effusion; an

Signs and symptoms, n = 105	**Frequency**	**Percentage**
Fever	105	100%
Headache	56	53.33%
Asthenia	48	45.71%
Adynamia	48	45.71%
Arthralgias	46	43.80%
Myalgias	43	40.95%
Abdominal pain	40	38.09%
Rash	39	37.14%
Vomiting	35	33.30%
Mucosal bleeding	31	29.52%
Retro-ocular pain	29	27.61%
Hepatomegaly	11	10.47%
Petexhiae	9	8.57%
Hypotensión	4	3.80%
Hypertensión	2	1.90%

Table 1.
Signs and symptoms observed in patients with dengue from 2018 to 2022 at the hospital den Niño Morelense (HNM) Mexico.

Laboratory Study	n	Frequency	Percentage
Thrombocytopenia	105	71	67.61%
Leukopenia	105	51	48.70%
Hemoconcentration	105	33	31.42%
Hypertransaminemia	61	44	72.13%
Hypoalbuminemia	45	15	33.33%
Coagulopathy	62	19	30.64%

Table 2.
Laboratory changes reported in HNM patients with dengue in the period 2018–2022.

abdominal x-ray or an abdominal ultrasound would help us determine the presence of ascites [12–14].

In a pediatric hospital, in the state of Morelos HNM (Hospital del Niño Morelense) in Mexico, a study was carried out from 2018 to 2022 of patients diagnosed with dengue, 105 patients were obtained, finding the following results, the average age was 9 years with DS + -4.1 years; 61% of the patients only presented dengue fever in the febrile phase, while 38% presented symptoms that placed them in the critical phase, and only 1% presented shock data; 100% of the patients presented fever; headache 53.3%; asthenia and adynamia in 45.71% and alarm data, such as abdominal pain, in 38.09%; 33.3% vomited and 10.47% reported hepatomegaly, 29.52% reported bleeding in the mucous membranes, 8.57% petechiae, and 1.9% hypotension (**Table 1**).

The first changes in the laboratory that can be detected in the febrile phase are leukopenia, thrombocytopenia, and increased transaminases. In the critical phase we find increased hematocrit, hypoalbuminemia, prolongation of coagulation times. In the HNM study, laboratory determinations were also performed on the patients, and thrombocytopenia was found in 59%, hemoconcentration in 31.42%, leukopenia in 48.7%, increased transaminases in 72.13%, and hypoalbuminemia in 33.3% **Table 2** [13].

Regarding complications in this series of HNM patients, pleural effusion was found in 5.71%, hemorrhage data in 4.76%, ascites in 1.9%, pneumonia in 0.95%, acute liver failure in 0.95%, and hypovolemic shock in 0.95%. No case of encephalitis was documented and 4.28% presented more than a complication.

3. Treatment

Treatment so far is symptomatic, and there is currently a dengue vaccine indicated for people with at least one primary infection, thereby reducing the risk of severe dengue.

According to the indications of PAHO, it is suggested to classify patients for treatment, as patients with ambulatory management, hospitalized patients for observation, and hospitalized patients for intensive management.

In the first group, patients present with fever, arthralgia, and myalgia live in an endemic area for dengue fever, which is why the diagnosis of dengue is suspected. If there is no evidence of dehydration or shock, fluids and fluids should be indicated. Antipyretics, if possible, should be reassessed every 48 hours; monitoring of alarm data, such as abdominal pain, vomiting, drowsiness, and bleeding data should be

indicated. At this stage, paracetamol 10–15 mg/Kg/dose can be used with a maximum dose of 4 g in 24 hours, in children, remember not to use salicylates, once the patient has been identified, it is advisable 48 hours after the onset of the symptoms to take a blood count to determine the hematocrit and platelet count [4].

Patients with any comorbidity, such as arterial hypertension, diabetes mellitus, asthma, hematological diseases, cardiovascular diseases, or some autoimmune disease, children under 5 years of age, pregnant patients, and patients at social risk (who have difficult access to hospitals) should be hospitalized for surveillance. For health services, it is important to maintain hydration, intravenous (IV) crystalloid solutions should be used in case of having a high hematocrit or if diuresis is <0.5 ml/kg/hr., insist on fluid intake and keep comorbidities under control. In these patients, hematocrit, platelet count, coagulation times (PT, PTT), DHL, and transaminases (ALT, AST) should be determined. In this phase, it is indicated to take serology to try to identify the serotype [10, 19, 20].

Patients who require shock management should start infusion of crystalloid solutions in a 20mlKg bolus to try to restore a mean arterial pressure according to their age, and management of colloid solutions should be evaluated according to the hemodynamic evolution. If a decrease in hematocrit is reported, hemorrhage should be suspected, so a transfusion of concentrated erythrocytes should be evaluated. If despite fluid management, the patient still has signs of hypotension, the use of inotropes should be evaluated. These patients, in addition to the laboratory tests that have been mentioned, tests should be taken to evaluate renal function, echocardiogram, chest, and abdominal X-rays in search of pleural effusion or presence of ascites and in case of neurological data, such as loss of state of consciousness or seizures, consider performing a head tomography, magnetic resonance imaging, and/or lumbar puncture; to evaluate his discharge he must be without fever, without data of hemodynamic alteration, normal platelet count, and normal hematocrit, as well as good tolerance to the oral route to be able to discharge him without risk of relapse [4, 9, 15].

It is a fact that after the COVID-19 pandemic, many things will change. In this case, we must not forget that both COVID-19 and dengue fever are viral diseases, or that they may share clinical characteristics, in addition to the fact that they must be to consider diagnostic possibilities when faced with a patient with fever or even not to forget that both infections can be present together. The diagnosis of dengue should be thought of as one of the traveler's diseases, so it is important to ask the patient about trips to dengue endemic places, and in this way, we can have cases of dengue fever in places where it is not endemic. The geographical and climatic conditions are not going to favor its spread, especially in communities where dengue fever is not endemic, however, it must not be forgotten that with changes in global climate conditions, the conditions for its spread can occur. Of dengue, as well as the appearance of different serotypes in regions where it is not common to find them [20].

4. Prevention

Prevention measures include the use of mosquito nets, avoiding the accumulation of scrap, and avoiding collections of stagnant water. Some authors have correlated the increase in urbanization with the increase in dengue cases, as well as the deficiency in the disposal of garbage from the communities; the use of repellents has modified the prevalence of the different serotypes, some studies reveal that people who have suffered from dengue fever will have better practices of preventive activities to avoid

contracting the disease again. After the pandemic, it was observed that in some places, they improved their hygiene habits in order to reduce the risk of contracting COVID, and that improved the health of people in dengue-endemic areas, it is suggested that in endemic areas the health authorities should send information to the inhabitants in order to improve health education in these areas [21].

It should not be forgotten that dengue fever is also related to seasonal weather variability, and it is a fact that global warming is causing changes in many regions of the world, so the spread of arbovirus infections may be modified [18].

5. Conclusions

Dengue is a health problem and children are among the vulnerable groups. The clinical picture may be mild, presenting only fever and general state attack, but it should not be ruled out that each patient diagnosed with dengue fever may present hemorrhagic dengue, shock and death. So, it is important to know the clinical picture, diagnostic methods, management and, above all, in endemic areas, continue with the prevention and eradication programs of the A. aegypti vector.

Conflict of interest

The authors declare they have no conflict of interest.

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Abbreviations

PAHO	Pan American Health Organization
DENV 1	Dengue virus serotype 1
DENV 2	Dengue virus serotype 2
DENV 3	Dengue virus serotype 3
ELISA	Enzyme-Linked ImmunoSorbent Assay
PCR	Polymerase chain reaction
NS1	Non-structural protein 1
IL12p70	Interleukin 12p70
IL6	Interleukin 6
TNF-α	Tumor necrosis factor alpha
IFN-γ	Gamma interferon
TNF	Tumor necrosis factor
HNM	Molerense Children's Hospital
PTT	Partial thromboplastin time
PT	Prothrombin time
DHL	Lactic dehydrogenase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase

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Chapter 3

Reemergence of Sylvatic Dengue Virus in Southern Senegal, 2021

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Abstract

As part of the syndromic surveillance of fever in Senegal, the virology department at Institut Pasteur de Dakar (IPD) in collaboration with the Epidemiology Unit and the Senegalese Ministry of Health conducted syndromic surveillance of fever in Senegal. Sample are from all suspected arboviral infections patients attending any of the sentinel sites. Collected blood samples were sent on a weekly basis at WHOCC for arboviruses and hemorrhagic fever viruses for screening of seven medically important arboviruses, including dengue virus (DENV). From January to December 2021, 2010 suspected cases were received among them 124 for confirmed to be DENV+ by RT-qPCR attempt of serotyping led to the detection of atypical DENV case from Sare Yoba area (Kolda region) which is unable to be correctly assigned to a serotype by the available tools (TIB Molbiol Modular Dx Dengue typing kit). Performed genome sequencing et phylogenetic analysis leads to the identification of a sylvatic DENV-2 strain closely related to a virus previously detected in Guinee-Bissau in 2009. This finding constitutes proof of the contemporary circulation of DENV-2 strain belonging to the sylvatic cycle in addition to well-known epidemic strains; this adds a piece of complexity to dengue management in Senegal. Alarmingly, it calls for improved genomic surveillance of DENV to know the genetic diversity of circulating strains in order to strengthen future vaccination policies.

Keywords: fever, syndromic surveillance, Sénégal, sylvatic DENV, réémergence

1. Introduction

In Africa, fever is the primary symptom that prompts patients to seek medical attention [1, 2]. The presence of a fever of unknown origin has historically been used as a starting point for treating malaria [3]. As malaria control efforts in Sub-Saharan African nations continue to yield positive results thanks to measure as large-scale implementation of malaria rapid diagnostic tests (mRDT), the incidence of this disease is decreasing, resulting in a smaller percentage of febrile illnesses attributed to malaria. During the period from 2000 to 2013, malaria mortality rates decreased by 47% worldwide and by 54% in Sub-Saharan Africa, which is the region most affected by the disease. This decline has resulted in an increase in the proportion of patients

exhibiting symptoms of non-malaria febrile illness (NMFI) [4]. Among myriad of pathogens such as viruses, bacteria, and parasites can cause acute febrile episodes indistinguishable from malaria.

Dengue fever (DF) is a viral illness caused by the dengue virus (DENV) etiological agent of the disease. The virus exists in four serotypes, namely DENV1–4 [5]. They belong to *flaviviridae* family and *flavivirus* genus. DENV is prevalent in numerous tropical and subtropical regions worldwide [6]. The virus is considered a significant public health threat in these regions due to its high morbidity and mortality rates [7]. Infections with DENV cause clinical manifestations ranging from self-limited flu-like symptoms, namely dengue fever (DF) to life-threatening infection associated with hemorrhage and or shock syndrome called severe dengue [8]. According to World Health Organization (WHO) estimates each year 390 million people are infected by the virus [9] with a case fatality rate ranging between 1 and 5% [10, 11]. In contrast to American and Asian countries, the virus epidemiology is not well known in Africa despite reports of the virus circulation since the nineteenth century [12, 13]. This underestimation in the African continent is linked to many factors as low awareness, lack of surveillance activities, the prevalence of pathogens associated with similar clinical manifestations, and the lack of reliable diagnostic tools [13].

In Senegal since 2011, in collaboration with the Senegalese Ministry of Health, the virology department and the epidemiological unit of Institut Pasteur de Dakar (IPD) set up a countrywide surveillance of influenza viruses and other respiratory tract infections associated viruses, namely 4S network [14]. This system was improved in 2015 to add the surveillance of other pathogens. Thanks to noticed increased number of febrile cases around the country not linked to malaria; the list of targeted pathogens includes arboviruses (Dengue, Zika, and Rift valley fever), bacteria, etc. [15]. Following years, this human sentinel surveillance throughout fever permitted the isolation and identification of many viruses, including DENV. In 2017, Dieng and colleagues [16] implemented genomic surveillance of DENV in Senegal throughout the 4S network collected samples. This allowed the detection and mapping of molecular characterization of DENv serotypes/genotypes circulating around the country [16]. DENV serotypes are maintained in two different ecologically and evolutionary distinct transmission cycles, namely the human cycle and the sylvatic cycle. The human cycle is sustained exclusively between humans and domestic or peridomestic mosquitoes, while the sylvatic cycle involves arboreal mosquitoes and nonhuman primates [17]. Although sylvatic strains of DENV play a pivotal role in the evolution and emergence of the virus, there have been no documented cases of ongoing and uninterrupted transmission [18].

In Senegal, particularly in the southern region of the country (i.e., the Kédougou area), the predominance of sylvatic cycles has historically played a significant role in the spread of DENV [19]. Since 2009, there have been numerous reports of dengue epidemics in Senegal, all of which have been associated with the epidemic cycle. This chapter discusses the reemergence of contemporary sylvatic DENV-2 strain in Southern Senegal, thanks to implemented genomic surveillance and 4S network system.

2. Material and methods

2.1 4S network sentinels sites for fever surveillance

In Senegal, a Sub-Saharan African country, a surveillance system for febrile illnesses has been in place for a long time. The Senegalese Ministry of Health, the

WHO country office, and the Institut Pasteur de Dakar (IPD), which hosts the WHO Collaborating Center for Arboviruses and the National Influenza Center, partnered to establish a febrile illnesses surveillance network [20]. The system initially monitored virological surveillance of Influenza-like illnesses (ILI) but was later revised with the establishment of the Senegalese Syndromic Sentinel Surveillance Network (4S network) based on a syndromic approach centered around fever. The 4S network is accountable for monitoring febrile illnesses at 20 sentinel sites across 14 administrative regions in Senegal, where population-based surveillance for ILI and other priority public health syndromes, such as malaria, dengue-like syndromes, and diarrheal syndromes, are conducted. Outpatient visits are enrolled and distributed across various regions of the country [21].

2.2 Sample collection

Clinical samples were collected from 22 sentinel sites around the country. For each suspected/ case that meets inclusion criteria, whole blood samples were collected using dry tubes and stored at +4 until shipping to the reference lab located at the virology department at IPD.

2.3 Sample shipping to reference lab

On a weekly basis collected suspected arboviral samples are shipped with epidemiological and demographic forms at the virology lab based at Institut Pasteur de Dakar. At the lab, samples were identified and a unique number of six digits is provided.

2.4 Sample handling and RNA extraction

Briefly, dry tubes were centrifuged at 2000 rpm for 5 minutes and the serum was harvested on cryotubes and then stored at – 80 for biobanking purposes. For the purpose of molecular screening, RNA extraction was performed from 140 μl of serum using Qiagen viral RNA mini kit (Qiagen, Hildan, Germany), according to the manufacture's recommendations. RNA is eluted on 60 μl of molecular grade water and stored on ice until further use.

2.5 RT-PCR diagnostic assays

2.5.1 panDENV detection

RNA was detected using Lightmix 1 step (Roche). Master mix for virus detection was prepared according to the table (**Table 1**) using a set of primers targeting DENV 3'-UTR region previously described by Wagner and colleagues [22]. The real-time PCR assay was performed using a CFX96 thermocycler (Biorad, France). The thermal profile used is described in **Table 1**. Any DENV RNA with Ct values below 32 was considered positive.

2.5.2 DENV serotyping assay

In the case of panDENV positivity, same RNA was systematically subjected to RT-qPCR to determine the associated DENV serotype using TIB Molbiol Modular Dx Dengue typing kit (cat. no. 40–0700-24; TIB Molbiol, Berlin, Germany) [19]. Using

Reagents	Volume	Step	Condition
Lightmix enzyme mix	9.5	RT	55° - 10mn
Forward primer	0.8		95° - 1 mn
Reverse primer	0.8	40 Cycles	95° - 15 sec
Probe	0.4		60° - 30 sec
Grade water	3.5		

Table 1.
Mixture preparation and conditions for RT-qPCR detection of DENV.

Reagents	Volume	Step	Condition
Lightmix enzyme mix	10	RT	55° - 10mn
PSR (*Dengue typing primer and probe*)	0.5		95° - 1 mn
		40 Cycles	95° - 15 sec
			60° - 30 sec
Grade water	4.5		

Table 2.
Mixture preparation and conditions for RT-qPCR DENV serotyping.

different probes serotype-specific and labeled with different fluorophores, the system allows discrimination of serotypes from 5 µl of RNA input. Surprisingly, at the end of the reaction used system fail to define the serotype of DENV+ samples collected from Sare Yoba in the Kolda region in 2021 (**Table 2**).

2.6 Sequencing of NS5 gene using nanopore sequencing

Using a set of primers FU1/FD3 specific to the flavivirus genus and previously described by Kuno and colleagues [23] we amplify ≈ 1 kb of NS5 gene. Obtained amplicons were visualized on agarose gel and then purified at 1:0.8 ratio using.

AMPure beads (Beckman Coulter Inc., Brea, CA, USA). Purified DNA was subjected to library preparation and sequencing using Oxford Nanopore MinION (Oxford Nanopore Technologies plc, Oxford, UK). The Rapid barcoding kit (SQK RBQ110.96), which uses a transposase-based barcode binding was used during library prep steps. The prepared library was loaded onto the R9 flow cell and a sequencing reaction was performed MinION MK1C device. After 24 hours of run, the raw data were collected on flash drive; base called was performed using guppy (https://community.nanoporetech.com) to generate fastq files. Bioinformatic analysis was performed using in-house script; Nanofilt (10) was used to trim barcode adapters (options -headcrop 50 and -tailcrop 50). Minimap 2 was used to map reads to DENV-2 reference genome (NC_001474.2) (11). Finally, generated consensus was subjected to National Center for Biotechnology Information (NCBI) BLASTn, which shows 99.66% identity with sylvatic DENV-2 (JF260983).

2.7 Development of specific sylvatic DENV-2 primer scheme

Since NS5 gene sequence provides a partial overview of virus genetic makeup based on the result from BLAStn using this gene. We downloaded full genome

sequences of closely related sylvatic DENV-2 sequences. Obtained dataset (n = 16) was aligned using MAFFT [24] and manually curated using geneious prime (Biomatters, New Zealand). Tilling PCR primal scheme was designed using the web-based tool (https://primalscheme.com/), and parameters sets to generate amplicons of around 900 bp and covering the coding region of sylvatic DENV-2 strains. Designed primers were synthesized generated by TIBMolBiol (Berlin, Germany), according to the manufacturer's recommendations.

2.8 Sequencing of full coding DENV polyprotein using nanopore technology

Amplicons were generated using Q5® High-Fidelity 2X Master Mix (New England Biolabs, Ipswich, MA, USA), according to a protocol previously described by Dieng and colleagues [25]. Briefly, primers were organized in two separate pools and then were used to generate overlapping fragments covering the full coding region of the detected sylvatic DENV-2 strain. Raw data were collected after 24 hours of sequencing and data analysis procedures were identical to those previously employed for NS5 gene sequencing.

2.9 Phylogenetic reconstruction

In order to establish and contextualize the evolutionary history of detected DENV strain, from Genbank database we downloaded representative sequences of described genotypes of DENV-2 and then aligned the resulting dataset using MAFFT [24]. We constructed a maximum likelihood (ML) tree using IQ-TREE [26] and then plotted the resulting phylogenetic tree and its associated metadata as years of sampling and host using R statistical software (version 3.6.0.).

2.10 Data management and statistical analysis

Patient information and results were recorded in a database, including patient ID, date of sample collection, and laboratory results. Weekly, the database was sent to Senegalese Ministry of Health for case notification and epidemiological report. Graphs were performed using the R statistical software (version 3.6.0.).

3. Results

From January 2021 to December 2021, 2010 blood samples suspected of arboviral infections were received at the WHOOC for arboviruses and hemorrhagic fever viruses. Samples were tested for DENV by RT-qPCR; among them, 123 shows dengue positivity. The algorithm for laboratory testing for DENV is presented in **Figure 1**.

The highest number of RT-qPCR DENV+ samples were recorded during the months of October and November with 53 and 63 confirmed cases, respectively (**Table 3**). At the serotype level, how most of the detected DENV-positive samples are DENV-3, followed by DENV-1, and finally DENV-2.

Untypable strain from Sare Yoba (Kolda region) was successfully sequenced using the proposed workflow (**Figure 2**).

Indeed, designed primers allow us to retrieve the nearly complete genome of the previously untypable DENV strain. Blast analysis shows that the strain is closely related to the sequences with accession number JF260983. The strain obtained during

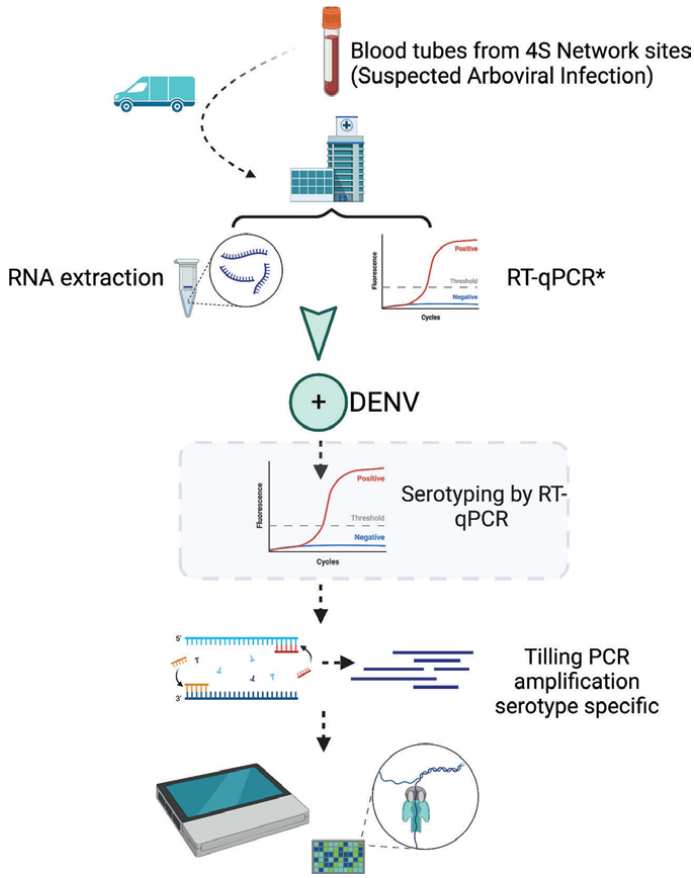

Figure 1.
Diagnostic algorithm for DENV molecular testing and serotyping.

Year	Month	Suspected	Rt-qPCR+	IgM+
2021	Jan	70	1	1
2021	Fev	69	1	0
2021	Mar	54	0	0
2021	Apr	90	0	0
2021	Mai	121	0	0
2021	Jun	118	0	0
2021	Jul	149	0	0
2021	Aug	146	0	0
2021	Sep	198	1	2
2021	Oct	355	53	13
2021	Nov	562	63	22
2021	Dec	78	4	4

Table 3.
Samples tested for DENV and lab results from january, 2021 to December, 2021.

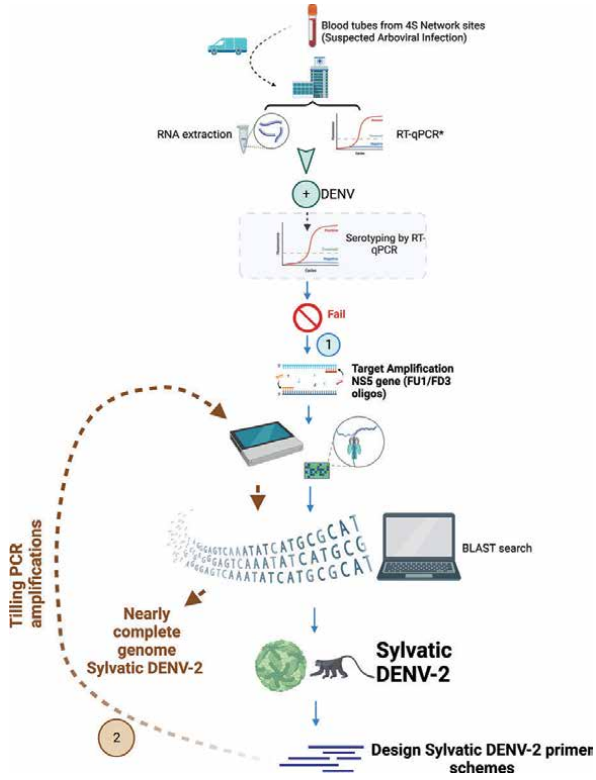

Figure 2.
Used workflow to identify and sequence sylvatic DENV-2 strain in Saré Yoba area (Kolda, region).

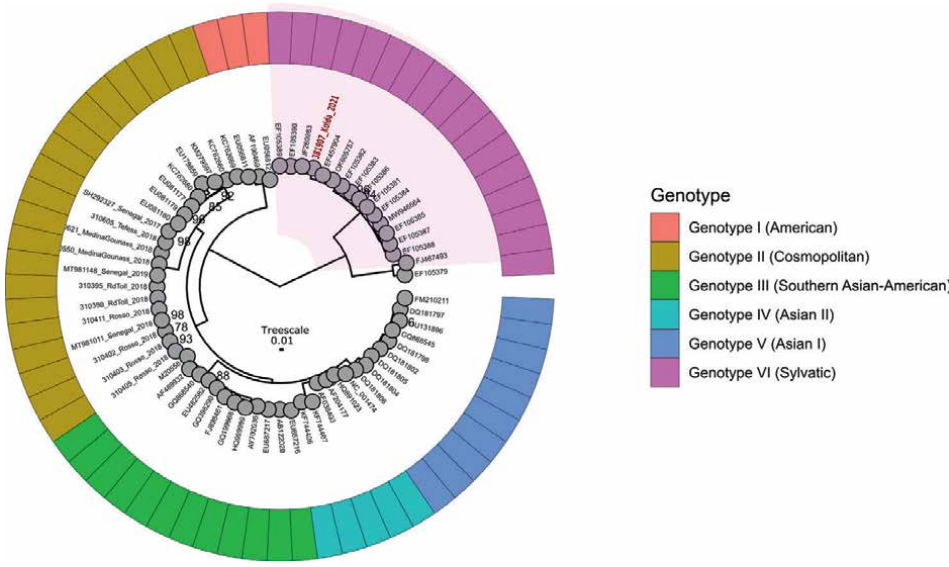

Figure 3.
Drawn maximum likelihood (ML) phylogenetic tree based on the nearly complete genome of DENV-2 sylvatic strain obtained during this study. The heatmap shows sequences genotype. The sequences obtained during this study are colored in red. The sylvatic genotype sequences are highlighted in light pink.

this work shares 99.66% identity nucleotide identity with the sequence JF260983. Genotyping of the sequences using the dengue typing tool shows the isolate cluster on the sylvatic DENV-2 group. This was confirmed by performing a phylogenetic analysis (**Figure 3**).

4. Discussion

This chapter presents findings from molecular surveillance of DENV conducted in Senegal in 2021 using the 4S network system, which allowed for the detection of the first cases of DENV during multiple outbreaks [14, 19]. From January to December 2021, 123 confirmed dengue cases were obtained out of the 2010 collected samples (**Figure 4** and **Table 3**).

The molecular surveillance of identified strains provided insights into the distribution of DENV serotypes/genotypes in Senegal [16]. We encountered a patient with an unusual dengue case in November 2021, and despite obtaining a high Ct value of 26.04 using the panDENV assay, we were unable to determine the virus serotype. Using the designed workflow (as shown in the figure), we were able to detect the presence of contemporary sylvatic DENV-2 strain circulating in Sare Yoba, located in the Kolda region. This paper presents the latest report on sylvatic DENV virus in Africa, and the first detection of circulating sylvatic DENV-2 in Senegal since 2000 [27]. The Kolda

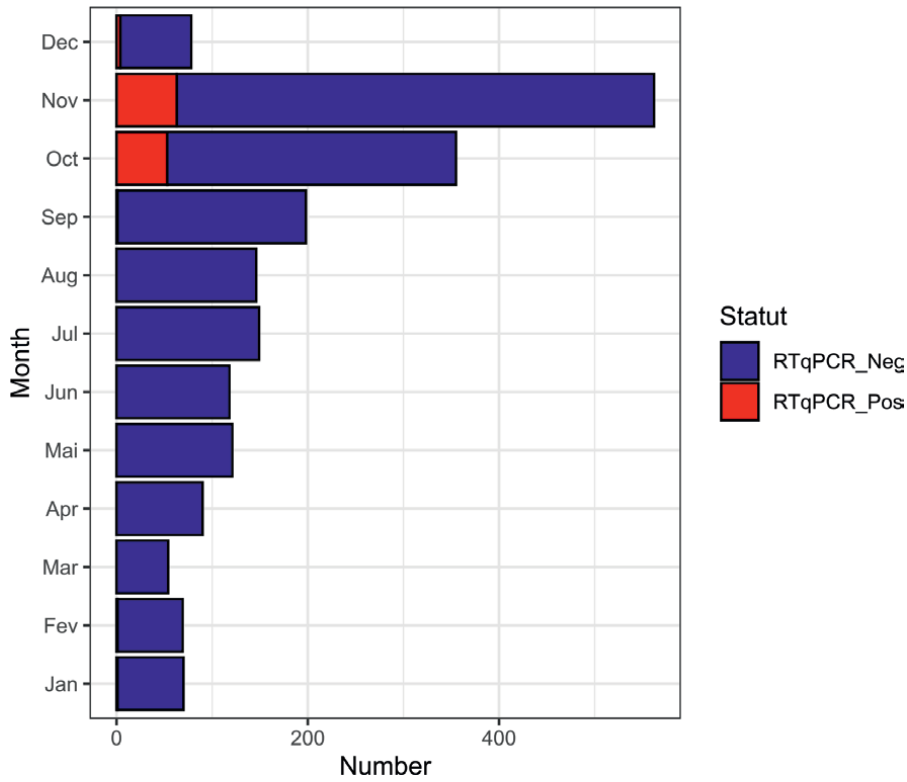

Figure 4.
Number of cases per months. The horizontal bar plot shows the number of people that were tested every month from January 2021 to December 2021. The red and blue bars represent positive and negative cases, respectively.

region, where the contemporary sylvatic DENV-2 strain was identified, shares a border with the Niokolo-Koba National Park, a habitat of monkey species, such as *Papio papio* and *Erythrocebus patas*, which serve as reservoirs for sylvatic DENV [17, 28]. Moreover, experimental studies using surrogate human models and cultured cells have indicated that the emergence of sylvatic DENV in human populations may not have a significant adaptive barrier, possibly due to the virus's opportunistic nature and ability to infect a diverse range of primate species [17].

In the context of utilizing genomic epidemiology to inform health policies, we have developed a user-friendly workflow for obtaining almost complete genome sequences using nanopore sequencing in less than 24 hours. Our generated Maximum Likelihood (ML) tree (**Figure 3**.) indicates that our strain, based on its near-full genome sequence, belongs to the West African DENV-2 sylvatic genotype and is closely related to a strain associated with hemorrhagic DENV found in a tourist who traveled to Guinea-Bissau *via* Senegal in 2009 [29]. This finding suggests that our strain is not related to the DENV-2 cosmopolitan genotype, which caused the most recent DENV-2 epidemic in Senegal [16, 25] highlighting a reemergence of sylvatic DENV-2 in southern Senegal.

The lower number of samples collected from the Kolda region in the 4S network suggests a potential underestimation of the DENV burden in this area. This suspicion was corroborated by the discovery of IgM-positive cases during a seroprevalence study in Senegal in 2021 (Unpublished data). Given the high suspicion of dengue circulation in the southern region, a "One health" approach is urgently needed, encompassing human, nonhuman primates, and vectors. This approach can enhance dengue fever surveillance *via* existing human malaria-like illness surveillance within the 4S network. Real-time genomic surveillance of DENV could be instrumental in discriminating between sylvatic and epidemic strains and improving virus surveillance across the country, with complex transmission dynamics involving both urban and sylvatic DENV cycles. Developing portable mobile platforms for epidemic virus surveillance in resource-poor regions is crucial, and lessons learned from previous epidemics, such as the Ebola outbreak and the SARS-CoV-2 pandemic, will enable better management of future epidemics and improved genomic surveillance of pathogens with epidemic potential.

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Conflict of interest

No conflict of interest for any of the authors was declared.

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Chapter 4

Asymptomatic Dengue and Silent Transmission

Pavithra Dilakshini Dayananda and B.G.D. Nissanka K. de Silva

Abstract

With over 90% of infected proportions being asymptomatic to dengue, their possible contribution to silent transmission has generated much attention in epidemic and non-epidemic settings. The challenges in identifying the true asymptomatic representation, owing to no clinical symptoms, have limited scientific knowledge of the asymptomatic dengue, its viral kinetics, immune mechanisms and underlying protective mechanisms in action. The chapter gives an overview of dengue, and its asymptomatic counterparts. It elaborates on the current knowledge in immunity, and immunopathology in symptomatic cases and provides postulations on possible protective mechanisms responsible for the asymptomatic nature of individuals. The chapter further discusses the importance of identifying the asymptomatic proportion in a community and the challenges in diagnosis. It highlights the major role, that asymptomatic carriers play in silent transmission, and its implications and further discuss the possible measures to minimize the transmission risk.

Keywords: dengue, dengue without symptoms, asymptomatic, dengue transmission, silent transmission, transmission risk

1. Introduction

Dengue is considered the most prevalent arthropod-borne viral disease in the world, causing more than 90 million cases and approximately 40,000 deaths per year [1, 2]. Causative agent- Dengue virus (DENV) is a single-stranded RNA virus of the Genus Flavivirus, which is comprised of 4 closely related, antigenically discrete serotypes, DENV1, DENV2, DENV3 and DENV4. However, in 2013 a 5th DENV serotype (DENV5) also has been reported [3]. DENV is transmitted by *Aedes* mosquitoes, mainly *Aedes aegypti* and *Aedes albopictus*. The virus and its vectors are widespread in over 100 countries worldwide, both tropical and subtropical [4]. Since there is no specific medication other than clinical management, the prevention of the disease relies mainly on vector control, and vaccine development is urgently required. Currently, a live attenuated vaccine, chimeric yellow fever 17D—tetravalent dengue vaccine (CYD-TDV), has been licensed for clinical use in some countries, and many candidate vaccines; including live attenuated vaccines, inactivated vaccines, recombinant subunit vaccines, viral vectored vaccines, and DNA vaccines are still under research and development [5].

Many factors have contributed to the expansion of dengue spread such as population growth, urbanization, inadequate water management, poor waste management, lack of effective mosquito control and increased global travel. Changes in global climatic patterns are believed to have expanded the vector habitat range and resulting increased epidemic activity may have caused an increase in the rate of viral genetic change and the emergence of strains or genotypes with greater epidemic potential [6–8].

Dengue has a wide spectrum of clinical outcomes ranging from asymptomatic to symptomatic; resulting in asymptomatic infections, undifferentiated fevers, Dengue Fever (DF), Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) [1, 9]; alternatively, they can be classified as dengue with warning signs (DWS), Dengue without warning signs (DWOS) and severe dengue (SD) as suggested by WHO [10]. Each year, 390 million DENV infections occur globally and an estimated 300 million result in asymptomatic/mildly symptomatic [11–14].

Primary Dengue infections are often observed to be asymptomatic and are known to generate immunity to the homologous DENV strain. However, 90% of DWS are known to reportedly occur following a second exposure to a heterologous strain of DENV [15]. It has been observed that sequential or secondary DENV infections are more likely to produce severe diseases [16, 17]. Cross-protection from previous infections and the neutralizing antibody levels also seem to play a major role in determining the level of severity of the disease [18, 19].

2. DWOS; asymptomatic infections

The incidence of dengue infection has been rising over the last five decades [20] and the majority of infected individuals are known to have no or insufficient symptoms to result in clinical presentation [12, 14]. Mildly symptomatic or subclinical infections are mostly referred to as DENV infections without major symptoms requiring medical attention, while the patients who have virologically or serologically confirmed dengue with no reported or detected symptoms are named dengue 'asymptomatic' patients [12].

Although asymptomatic infections are considered more frequent than symptomatic, the relative number of cases is observed to be vary according to the year of infection, geographical area, the epidemiological context, the immunological status of individuals, and the circulating serotypes and viral strains [21]. The ratios of asymptomatic to symptomatic cases have been shown to vary from 2.1:1 to 13:1 around the world [22] making this change in the proportion of symptomatic to asymptomatic infections, one of the main contributors to the rise in the dengue incidence.

A study carried out in Thailand during 1980–1981 reported a ratio of asymptomatic to symptomatic cases as 6.1:1, while this ratio was refined as 5.5:1 for DENV 1 cases, 4.5:1 for DENV 2 cases and for entirely asymptomatic for DENV4 cases [16]. During the period of 1998–2000, a similar survey in Northern Thailand reported a ratio of 1.1:1 [23]. A study carried out in Central America reported a ratio of 13:1 during the period of 2001–2003 when DENV2 was dominant and a ratio of 6.1 when DENV1 was frequent [24]. It has been reported that the Health authorities in Singapore assumed ratios between 2:1 and 10:1 during the period of 2006–2007 [25]. Studies from Sri Lanka have shown that the asymptomatic to symptomatic ratio was 3.4:8.4 between 2008 and 2010 [22]. In a pediatric dengue cohort study in Nicaragua, a wide variation from 16.5:1 in 2006–2007 [26] to 1.2:1 in 2009–2010 [27] has been shown. All these studies reveal the extensive variation of asymptomatic to

symptomatic ratios, where the differences might be attributed to the extrinsic and intrinsic factors of the host and the circulating virus types [24, 28].

Clinically apparent dengue is frequently studied with various research objectives. However, the science behind asymptomatic dengue is inadequately investigated mainly due to the challenges in diagnosing asymptomatic cases on time. Thus, understanding the host factors such as the role of immunity on the lack of clinical symptoms and or the other protective mechanisms for being symptomless has mainly been based on careful investigation of symptomatic cases.

2.1 Dengue; immunity

Once an immune-susceptible host meets with the infection, an acute, self-limiting febrile systematic syndrome is known to develop usually within initial 4–7 days. This is known to be associated with strong innate and adaptive immune responses [29].

2.1.1 Innate immune response

At the site of the mosquito bite, Langerhans cells, dermal cells and interstitial dendritic cells of the innate immune system become the initial targets for DENV [29]. The three cell types, monocytes, macrophages, and dendritic cells which are tolerable for DENV infection act as the main phagocytic cells of the innate immune system, responsible for detecting and removing hostile pathogens. These three major phagocytic cells also function as antigen-presenting cells critical for the initiation, expansion, and polarization of adaptive cellular immunity [30]. All these innate immunity mechanisms are triggered immediately upon pathogen invasion and play a significant role in managing pathogenic infection. The killing of target cells is associated with inflammatory cytokine and or chemokine responses [30]. However, DENV has evolved successfully to suppress innate immunity and to infect the host productively using passive and active evasive strategies, which have a negative effect on the subsequent production of antigen-specific adaptive immunity to these viruses [31].

2.1.2 Adaptive immune response (cell-mediated immune response)

Many studies have shown that the adaptive immune response to DENV has protective as well as detrimental aspects [32]. Dengue-induced immune enhancement plays a major role in the clinical manifestations of dengue disease. The imbalanced and deregulated, cell-mediated immunity is considered as a major component in severe dengue conditions [29]. It is hypothesized that DENV infection of monocytes and macrophages increases T cell activation, leading to the release of cytokines and chemical mediators such as Tumor Necrosis Factors (TNF), Interleukins (IL), Platelet Activating Factors (PAF), complement components and histamines causing increased vascular permeability, plasma leakage, shock and malfunction of the coagulatory system resulting in hemorrhage and shock [33, 34]. In this phenomenon, DENV infection of dendritic cells strongly activates CD4+ and CD8+ T cells which produce a surplus of cytokines, which recruit numerous other cytokines and chemical mediators that further increase the vascular permeability of the host [34, 35].

2.1.3 Adaptive immune response (humoral response)

Individuals infected with DENV generate serum antibody titers that provide long-term protection against future homotypic infections with the same serotype [32].

Infection with DENV also builds a degree of cross-protective immunity against the other three DENV serotypes by means of heterotypic (cross-reactive) IgG antibodies which usually persist for a duration of several months to a few years [36]. The produced heterotypic antibody titers are known to reduce over long time periods of approximately 4 to 20 years [37]. However, conversely, the homotypic IgG antibody titers are known to increase over time which could be due to the preferential survival of long-lived memory B cells producing homotypic antibodies [37].

2.2 Dengue; immunopathogenesis

The postulated hypotheses on dengue immunopathogenesis include the antibody enhancement theory, cross-reactive memory T cells activation and the original antigenic sin where all in a way cause either an overproduction or a skewed profile of cytokine release [38, 39].

Antibody-dependent enhancement (ADE); The leading hypothesis is that DHF occurs via ADE of a DENV infection [40–43]. Preexisting heterologous, cross-reactive antibodies from a previous infection (or maternal antibodies in infants) recognize and bind to heterologous DENV in a secondary or tertiary infection (primary infection in infants), are unable to neutralize this virus, either because they are non-neutralizing, or due to inadequate avidity or occupancy [41, 44–46]. These non-neutralizing antibody–virus complexes are known to increase the infection of monocytes via their Fc receptors, dramatically increasing viral replication and load, thereby causing DHF. Weak and non-neutralizing cross-reactive antibodies induced from immunodominant B cell epitopes are known to comprise the majority of the humoral immune response to DENV infection [38, 47, 48].

Cross-reactive memory T cells activation and Original antigenic sin; the second hypothesis indicates that there is a highly skewed cellular response to heterologous DENV infection motivated by low affinity, cross-reactive memory CD4+ and CD8+ T cells [49–51]. Cross-reactive T cell epitopes have been identified across the DENV proteome, however, immunodominant CD8+ T cell epitopes in dengue non-structural protein NS3 have been found strongly associated with DHF [15, 52, 53]. The common theme throughout DENV immune enhancement is the concept of "original antigenic sin," which describes the shift in the hierarchy of immunodominance that occurs when previous exposure to cross-reactive antigens alters and inhibits the subsequent immune response to related antigens, either as a new infection or by vaccination [49]. Both humoral and cellular responses are known to be plagued by such misdirected or inappropriate heterotypic immunity [54].

All these mechanisms are known to increase the activation of immune cells, resulting in impaired immune responses or cytokine storms that cause endothelium dysfunction and increase vascular permeability. However, multiple host and viral factors seem to be influencing the determination of the disease severity of DENV infections via favorable and unfavorable interactions thus have triggered much research interest [55].

2.3 DWOS; asymptomatic infections - protective mechanisms

Fundamental immunological differences in the immune responses associated with symptomatic and asymptomatic infections have been studied [56, 57]. The kinetics of asymptomatic infections are known to differ from the symptomatic infections in the magnitude of the viremia and the rate of clearance [58]. The protective mechanisms that contribute to the lack of clinical manifestations in individuals, that remain asymptomatic

or inapparent compared to symptomatic dengue cases have been found interesting and are still being investigated. Epidemiological risk factors such as age, duration between consecutive dengue infections, DENV serotypes of previous infections, concentrations of pre-existing heterotypic neutralizing antibodies, interactions between viral genotype within the serotype and resulting immune responses are known to be associated with these subclinical outcomes after the dengue infection [12, 23, 59–61].

In general, it is regarded that secondary infections are associated with more severe disease due to the phenomenon of ADE and/or cross-reactive T cells [62]. However, post-secondary infections are known to induce different immune responsiveness in susceptible hosts and have also been found to impact upon inapparent rates [63]. Thus, the role of previous infection in perhaps decreasing or increasing the risk of infection causing it inapparent needs to be further investigated [12, 64]. Alexander et al. (2020) have studied the impact of frequent immune boosting; that occurs as a result of frequent disease exposure in dengue-endemic areas, on the fluctuating symptomatic and asymptomatic ratios [28]. It has been reported that antibodies play a greater role than immune cells in heterologous DENV infections [65]. Neutralizing antibodies seem to play a major role in this and it is evident that, the individuals who are previously exposed to DENV, manifest clinical symptoms differently due to the presence of pre-existing neutralizing antibodies resulting in asymptomatic or inapparent dengue status on many occasions. Furthermore, high concentrations of neutralizing antibodies against DENV infection have been frequently observed in asymptomatic individuals [61, 66–68].

Variations in immune reactions to the virus have been reported in dengue asymptomatic and symptomatic patients. In a study carried out by Simon-Loriere et al. (2017), the inflammatory pathways and innate immune responses were found similar in asymptomatic and symptomatic diseases. However, the expression of proteins related to antigen presentation and subsequent T and B cell activation pathways were found differently regulated, independent of the viral load or previous DENV infections. Asymptomatic individuals have been found to have increased T cell responses with feedback regulation compared to symptomatic counterparts [57]. According to his findings, asymptomatic infections seem to be determined by increased activation of the adaptive immune response and properly controlled mechanisms leading to the removal of viral infection without excessive immune activations [57].

Furthermore, apart from immune status, host genetic factors are considered to have an impact on the protective mechanisms in asymptomatic diseases [56], which involves a complex network of genes that are expressed differentially in the asymptomatic or inapparent individuals. A polymorphism in Fc gamma receptors (FcgRIIA) has been found to be associated with inapparent infections compared to symptomatic infections with DF or DHF in the Cuban population [56]. Moreover, according to the studies of [67] a broad down-regulation of host defense response (innate, adaptive, cytokines and matrix metalloprotease) genes in asymptomatic individuals against symptomatic patients. A selective up-regulation of distinct genes which are associated with protection has been observed [66]. However, these observations warrant further investigations in order to correlate their expression with conferring protection against clinical dengue infections.

2.4 DWOS; asymptomatic infections- detection

Detection of DWOS or asymptomatic infections is known to be challenging. The symptomatic dengue can be clinically suspected based on the symptoms and

a confirmatory laboratory diagnosis will provide a definite diagnosis. Detection of asymptomatic cases happens only based on laboratory diagnosis, since there are insufficient or no clinical cues for infection [68].

Direct diagnostic methods such as molecular and antigen-detecting methods are not usually considered convenient to detect asymptomatic infections owing to the shorter period of viremia after the infection. Serological tests such as HAI, ELISA and PRNT have been accepted as suitable methods to detect DWOS and have been frequently applied in detecting asymptomatic dengue cases than the direct diagnostic methods [68]. However, direct methods to detect acute infection and indirect methods; mostly the serological methods and further, mosquito inoculation techniques have also been incorporated in many studies for detecting asymptomatic infection in high-risk cohorts (**Figure 1**) [69–71].

Surveillance studies for DWOS or asymptomatic infections are carried out in the general population over a long period of time with frequent blood sampling and testing [16, 23] and by screening the dengue high-risk groups [71].

2.5 Dengue transmission

Transmission of DENV among human hosts occurs through horizontal and vertical transmission pathways. In horizontal transmission, viruses are transmitted among individuals of the same generation. Human-to-mosquito transmission is known as the most common mode of horizontal transmission, while transmission through blood transfusion [25, 70, 72–76] and organ transplants [25, 77] have also been infrequently reported. In addition to these transmission modes, a few cases of nosocomial transmission through needle stick injury and mucocutaneous exposure have also been reported [78, 79]. The difficulties in differentiating non-vector transmission from vector or mosquito transmission in dengue-endemic areas could be the result of the observed infrequency of records of these cases [80].

Studies have been carried out to investigate the possible sexual transmission of DENV. So far, cases of DENV in semen [81, 82], and vaginal secretions [83] have been

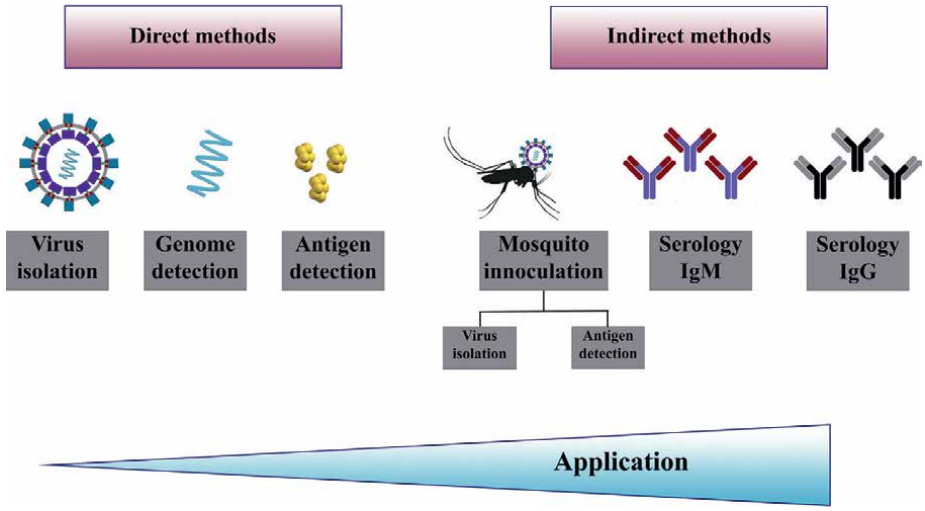

Figure 1.
Schematic diagram illustrating the application of diagnostic tests for the detection of asymptomatic dengue.

rarely recorded. However, in 2019, two cases of possible sexual transmission were reported in Spain and South Korea [84, 85]. Thus, though plausible, sexual transmission of dengue is considered extremely rare and uncommon in endemic communities [86].

Vertical transmission occurs when the virus is transmitted from mothers to their offspring, through intrapartum transmission [87–89] or transmission at the onset of delivery [80, 87]. Although DENV virus particles have been found in breast milk, the studies are insufficient to conclude the transmission of DENV via breast milk [90], however considering the benefits and immunological protection from breast milk to infants, breastfeeding in DENV-infected mothers are encouraged in dengue -endemic regions [91, 92].

2.5.1 Human to mosquito transmission of DENV

A susceptible female *Aedes* mosquito acquires a DENV infection after it consumes a blood meal from a dengue viremic person. When viremic blood reaches the mosquito midgut, the extracellular virus binds to undefined receptors on the cellular surface of the midgut epithelium. Once the virus is capable of successfully infecting and replicating in midgut epithelial cells, a new progeny of viruses is shed into the hemocoel, where it can, later on, disseminate and infect secondary tissues, legs, brain and salivary glands [93]. The duration of the viral incubation between the time of ingestion and reaching the salivary glands, where mosquitoes become infectious is known as the extrinsic incubation period (EIP), which is generally considered as 8–12 days [94]. Upon adequate viral replication in the salivary glands, the mosquito becomes a potential vector to transmit DENV to a new host during the next probing or feeding event [93].

The factors influencing the transmission of DENV from humans to mosquitoes include viral, host, vector and environmental aspects. In terms of host factors, viral titer in the human plasma and duration of human infectiousness are considered. The amount of viral titer circulating in the blood of an infected human influences the possibility of a mosquito becoming infected after a blood meal. Mosquito infectious dose, or the viremia in humans that is required to infect 50% of mosquitoes differs between viral serotypes [95]. A dose-response relationship is generally observed with an increasing number of DENV RNA copies [96]. The period between infection and the onset of infectiousness in a human is called the Intrinsic Incubation Period (IIP). The intrinsic incubation period of a human varies, and it is typically considered as 4–7 days [94]. It is estimated that onward transmission results from mosquitoes biting during the pre-symptomatic phase of DENV infections in most cases than, during the post-symptomatic period [14]. Further, it is also reflected that, patients with a high early viremia have a greater probability of having an extended duration of DENV infectiousness. Furthermore, host immune factors [96] and host stimuli for mosquito attraction such as body temperature, body odor, blood type [87, 97], etc. are also known to contribute as host factors for dengue transmission.

As for vectors, diurnal and crepuscular biting behavior of both *Aedes* mosquito species [98], anthrophonic nature, considerable flying span, and highly domesticated nature, especially of the primary vector, *Ae. aegypti* mosquitoes [99] have made them excellent vectors in disease transmission. Mosquito susceptibility to infection and vector competence (VC), which elaborates on mosquito infection, dissemination and onward transmission of the virus, plays a major role in transmission. Relative vector competence of two major vectors *Ae. aegypti* and *Ae. albopictus* have been extensively studied. *Ae. albopictus* are known, more susceptible to midgut infection than *Ae. aegypti*, but the ability to disseminate the virus of *Ae. aegypti* has been found greater

suggesting a greater potential for transmission in nature [100]. The susceptibility for DENV in mosquitoes of different geographical strains has been reported [101, 102] and population-specific differences in the susceptibility with each serotype, have revealed consistent patterns of high and low infection [103]. Further, differential susceptibility by different viral isolates of genotypes within the same serotype in a single geographical population has also been reported [101, 104, 105].

Dengue virus is also known to manipulate the biology and behaviors of the infected host to facilitate virus transmission [93, 106]. Studies on the blood-feeding behavior of DENV-infected mosquitoes have investigated the time duration of probing and feeding [107], transmission efficiency during probing [108], and motivation and avidity to feed [106, 109] and revealed the relationships of such in disease spread.

Environmental factors have been known to play a major role in dengue transmission via mosquito vectors [110]. The temperature has been known to have implications in altering mosquito VC to transmit viruses. The lower temperatures are known to induce slow virus replication and high temperatures are known to induce increased virus replication resulting in reduced EIPs [100, 111]. Changes in the humidity levels are also known to intervene with the vector competency of vector mosquitoes, which affect DENV transmission [110]. Research interest in the factors contributing to DENV mosquito transmission is ongoing and in-depth studies are warranted [93].

2.6 Silent transmission; vector and non-vector transmission

Studies on vector transmission of DENV from asymptomatic patients are rare and the level of mosquito infectiousness has not been adequately investigated [69]. It was long assumed that people with inapparent and asymptomatic infections fail to infect mosquitoes and have low viremia levels. Many studies have reported lower viremia in asymptomatic infections than those of symptomatic infections but also in detectable levels [58, 69, 112–115].

It has been shown that people with asymptomatic infections have had 100-fold lower infectious doses of viruses to mosquitoes that eventually have resulted in larger viral loads in infected mosquitoes [116]. This was also evident to us in a study carried out in Sri Lanka, where silent transmission from asymptomatic individuals (with no detectable viremia or sometimes no detectable antigen levels), to vector mosquitos (with detectable antigen levels) was observed [71]. A recent study has reported a slower viral decay rate in asymptomatic subjects compared to symptomatic individuals, enabling the asymptomatic cases more available for silent transmission [58]. Furthermore, studies to evaluate mosquito infectivity of asymptomatic subjects have shown a significant increase in mosquito infectiousness among asymptomatic cases than the symptomatic cases (**Figure 2**) [18, 69], postulating that strong immunological response and high cytokine levels during symptomatic illness reduce human infectiousness to mosquitoes in symptomatic dengue cases [69].

Non-vector transmission of DENV via atypical routes such as blood transfusion, organ transplant and intrapartum transmission has been confirmed in many studies, and the possibility of these transmission routes originating from asymptomatic, pre-symptomatic or subclinical cases has also been discussed [12, 73, 117–120]. Furthermore, vertically transmitted dengue in a neonate born to a mother with asymptomatic dengue infection has been reported in a recent case study from Sri Lanka, and instances, where such cases are misdiagnosed owing to no maternal history in asymptomatic mothers have been discussed [121].

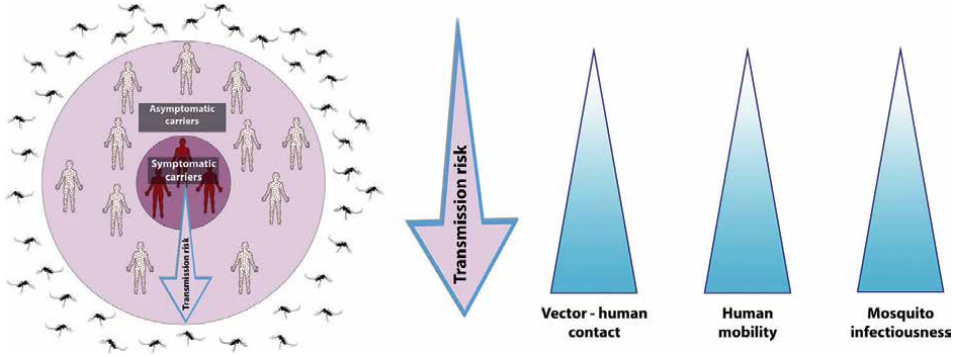

Figure 2.
Schematic diagram illustrating the increasing transmission risk towards the asymptomatic proportion of an infected community.

According to the modeling analysis of Bosh et al., (2018), it has been suggested that inapparent infections contribute appreciably to DENV transmission and its disease burden [14]. Further, their finding that approximately one-quarter of an individual's infectiousness occurs prior to symptom onset, supports the hypothesis that a large proportion of human to-mosquito transmission is silent [14]. Collectively, these evidences show that asymptomatic cases play a major role in silent transmission, having a high transmission risk compared to symptomatic cases (**Figure 2**). Furthermore, the fact that the vector- host contact is considerably high in asymptomatic carriers through their daily routines compared to symptomatic cases, who will be hospitalized or less accessible should also be accounted with great concern. In addition, human mobility is also known to play a key role in the spread [122, 123], thus silent transmission of dengue via undisrupted daily routings of these asymptomatic or mildly symptomatic carriers can be identified as a key factor contributing to the dengue spread than the symptomatic cases [69, 100] (**Figure 2**).

2.7 Seroprevalence and risk of antibody-dependent enhancement

Relatively high dengue seroprevalence among the dengue endemic communities around the world has been reported [13, 24, 124–129]. Comparative to the number of confirmed dengue cases, an increased level of dengue infection suggested by high IgG seropositivity in endemic areas, has revealed a vast majority of DENV infections [128]. Attributing to the fact that the majority of dengue infections in these communities are either asymptomatic or inapparent [11, 14, 16, 23, 65].

As a consequence of this, a considerable proportion of the population who are immune to a circulating dengue serotype/strain after an epidemic will be created. Co-circulation of several dengue serotypes in dengue-endemic areas has been reported [129, 130]. Worsening the situation, the prolonged seroprevalence in symptomatic and asymptomatic individuals has also been observed in studies [13]. Thus, this proportion would be at risk of developing ADE or severe dengue in a subsequent epidemic of a differing dengue serotype with non-neutralizing antibodies or neutralizing antibodies at sub-neutralizing levels (**Figure 3**).

Furthermore, the transmission of heterogeneous anti-dengue antibodies from symptomatic or asymptomatic cases through blood transfusion or organ transplant, and transmission of maternal antibodies to infants has also been suggested to enhance

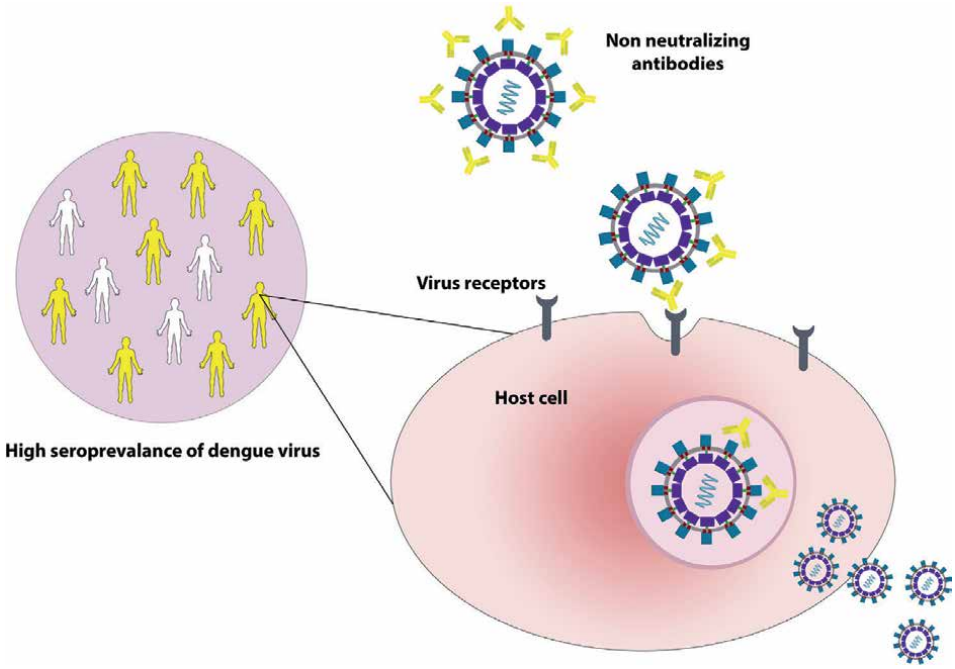

Figure 3.
Schematic diagram depicting the high seroprevalence of dengue virus in dengue-endemic communities and the risk of developing antibody-dependent enhancement during subsequent infections.

the viral infectivity and virulence in recipients who will later be exposed to a heterotypic DENV infection [131].

The global incidence of DHF/DSS has increased more than 500-fold in recent years [11]. And the risk of dengue virus or its antibodies which can be transmitted through such different passive modes of transmission has been identified as a major counterpart.

2.8 Prevention; a way forward

Prevention from silent transmission through mosquito vectors can only be achieved by vector control, in a setting where asymptomatic carriers can only be identified by certain laboratory identification techniques. However, the routing fogging and insecticide spraying practices just after a case report (peri-domestic space spraying) can be taken as an initial step to reduce the risk of transmission from asymptomatic carriers. Although many studies have suggested the importance of screening the populations for DENV, and dengue seroprevalence, no available efficient measures and or diagnostic services for such events prevail in many dengue-endemic countries [75].

However, there is a need to incorporate integrated approaches including increasing awareness among the community, establishing routine diagnostic methods for screening asymptomatic carriers and incorporating preventive measures to reduce the exposure, which will eventually help in reducing the dengue burden [75].

Many endemic countries have identified the risk of transfusion-associated transmission from asymptomatic donors, and have adopted policies where they recommend screening of blood products. The positive blood donors will be deferred for periods depending on the endemicity of the region. Similarly, transplant guidelines in

some countries have recommended dengue screening prior to transplantation and a specific deferral period before taking up for transplant surgery if the donor or recipient is found positive for dengue.

Vaccines and herd immunity; though seems like the only promising solution, limited knowledge of immune responses against dengue infection, lack of human or animal model of disease, and suboptimal assay strategies to detect immune responses after infection or vaccination, which are some barriers to the vaccine and drug development. Furthermore, in addition to the protection against symptomatic infection, it is also important to assess protection against asymptomatic infection.

3. Conclusion

Asymptomatic or inapparent dengue infections provide a fundamental link in the chain of disease transmission in dengue-endemic communities. The knowledge gap in understanding the viral kinetics of asymptomatic individuals along with their immunorespnoses must be urgently fulfilled and investigative studies on such should be encouraged. Understanding the presence and the prevalence of asymptomatic to symptomatic proportion of a community enables a glimpse of the targeted population and helps in introducing disease management strategies. The chapter highlights the increased transmission risk towards the asymptomatic carriers of the community, attributed to the increased vector-human contact, human mobility and mosquito infectiousness. All precautions must be taken to reduce dengue transmission in a community via vector and non-vector routes. Furthermore, the seroprevalence of a community must be routinely monitored and the vaccine efficacy in such settings depending on the endemicity, should be closely evaluated.

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Conflict of interest

The authors declare no conflict of interest.

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Chapter 5

Dengue Virus Surveillance and Blood Safety: A One Health Perspective

Festus Mulakoli, George Gachara, Eric Ndombi and Samoel Khamadi

Abstract

The provision of blood products to save a life is a noble undertaking for any organization tasked with the duty. In addition to saving millions of lives, blood products pose health risks associated with adverse events. Much has been done to mitigate these challenges, but emerging new infectious diseases pose a public health challenge to both the safety of blood and its availability. The dengue virus an arbovirus is one such virus that is endemic in tropical and subtropical countries. The data emerging from the published papers show that dengue could be a major threat to blood safety and availability in the future. To address these threats, a collaborative approach through one health system is the only avenue to provide a last solution. One health has been implemented as a strategy to mitigate zoonotic diseases and its results are very impressive. This piece of work is a fraction of our larger project that aims to address threats to the dengue virus and blood safety in Kenya and the rest of Africa. In conclusion, adopting one health in the fight against the dengue virus in blood safety will be the best approach to ensure a safer supply of blood products.

Keywords: dengue virus, blood safety, surveillance, one health

1. Introduction

Dengue fever is a mosquito-borne disease endemic in the tropical and subtropical regions of the world. The highest burden of diseases is reported in the Asian and South American regions [1]. The virus has four serotypes (DENV 1–4) that are antigenically different, but with variations in their immunological response. The dengue virus inhabited primates before jumping into the human population. It is one of the main arboviruses associated with frequent disease outbreaks reported annually in endemic regions [2]. The rapid spread of the dengue virus has had a negative impact on blood safety and availability in endemic areas. For example, there is a reduction in the number of suitable donors during DENV outbreaks, as shown in studies conducted in India, China and Brazil. Affected countries have different strategies available to mitigate these threats, but with minimal success. The challenge has always

been the lack of collaboration between entities involved in surveillance activities. The most critical limitation is the inability to share critical surveillance information on emerging disease patterns [3]. From an expert point of view, we believe that the adoption of one health in disease surveillance would be the best avenue to guarantee a safer blood supply.

One health, as is known, is a multidisciplinary platform where experts in human health, animal health, and environmental health work collaboratively to combat both human and animal diseases. These synergistic efforts put blood transfusion services in a better position to safeguard their blood supply in an era of the re-emergence of the dengue virus [4]. The Manhattan Principles, which outline relationships between infectious diseases, the environment, human health, and economic development activities, established the phrase "One Health" in 2003 [5]. This was after the outbreak of Ebola virus disease (EVD), a Filoviridae virus, in West Africa after the death of the great apes. Since then, high-level interest and acceptance of One Health initiatives have grown around the world [6]. The concept of One Health was adopted to allow the sharing of information and to foster collaboration between different sectors. It is a multidisciplinary initiative in which people working in different sectors within their countries, continents, and global regions come together to solve a common public health problem. The connectivity between humans, animals, and their ecosystems is agreed to play a significant role in the spread of infectious diseases [7–10]. This book chapter is a fraction of my Ph.D. project that seeks to highlight and evaluate the importance of adopting one health disease surveillance approach to safeguard blood supplies across the world. One aspect of one-health is helping different experts from different fields share information that can detect and suppress diseases upstream before they cause human disease [11].

2. Current evidence of transfusion-transmitted dengue

Transfusion-transmitted dengue (TTD) is a growing concern for many transfusion services in tropical and sub-tropical countries. These countries have experienced frequent outbreaks of dengue in the last ten years. The increasing number of dengue incidents increases the likelihood that blood components manufactured during dengue outbreaks could be infectious [12, 13]. The first documented cases of TTD in the literature were reported in two studies in Hong Kong and Singapore in 2008 and 2012, respectively. The cases involved blood recipients who received blood transfusions from asymptomatic blood donors. On evaluation of the transfused blood, the serotype detected in blood recipients was the same as the serotype present in blood donors. This became the first evidence of dengue transmitted by blood transfusions [14, 15]. Irrespective of this evidence, little was done to communicate this information to alert other regions where the dengue virus is endemic. With this evidence, it was important for all blood transfusion services in regions with frequent outbreaks of dengue virus to have taken urgent steps to secure their blood supply. This is a gap that needs to be filled by integrating one-health into our mitigation efforts to protect blood supply and availability.

In regions with widespread outbreaks of the dengue virus, the probability of receiving blood from asymptomatic donors is high and is easily missed by symptom-based exclusion criteria [16]. The high number of asymptomatic blood donors is

the principal cause of the rising incidence of reported TT-DENV cases in dengue-endemic regions [17–20]. Asymptomatic individuals have a higher viral load on their peripheral blood circulation, but they do not exhibit signs and symptoms associated with dengue fever. This makes it difficult for an experienced blood donor recruiter to exclude such risky donors. Things have also been made worse by a regional variation in the incidence rate of dengue viremia from voluntary blood donors. The case at this point is Brazil with 0.04–0.81%, Puerto Rico with 0.02–0.19%, and Honduras with 0.3% [21–24]. Data gathered a few years ago depict a viral viremia that can last up to 24 hours before the manifestation of any clinical symptoms [25]. Unfortunately, minimal information is available during outbreaks, making it difficult for BTS to select donors during recruitment [26].

The dengue virus is rapidly spreading to new areas and significant outbreaks are becoming more common. **Figure 1** illustrates the global region with documented cases of dengue viral markers detected in healthy blood donors. The highest burden of dengue among eligible blood donors is seen in Brazil and India [17, 20, 27–35]. The burden from other regions is lower, probably masked by a lack of testing and surveillance initiatives. The susceptibility to the virus has recently changed as individuals targeted for donation have become vulnerable. Most potential blood donors would be rejected if they were subjected to pre-donation screening [36]. This has affected the blood supply because more blood donors are deferred from donating blood due to dengue infection or exposure. Blood transfusions from asymptomatic viremic donors will also increase the probability of transmission. Although effective disease reduction and dengue screening techniques have been implemented in developed countries, the initiative is costly for low-income countries. As the number of patients with DHF/DSS increases, so will the demand for blood products such as platelet rich plasma [37–39].

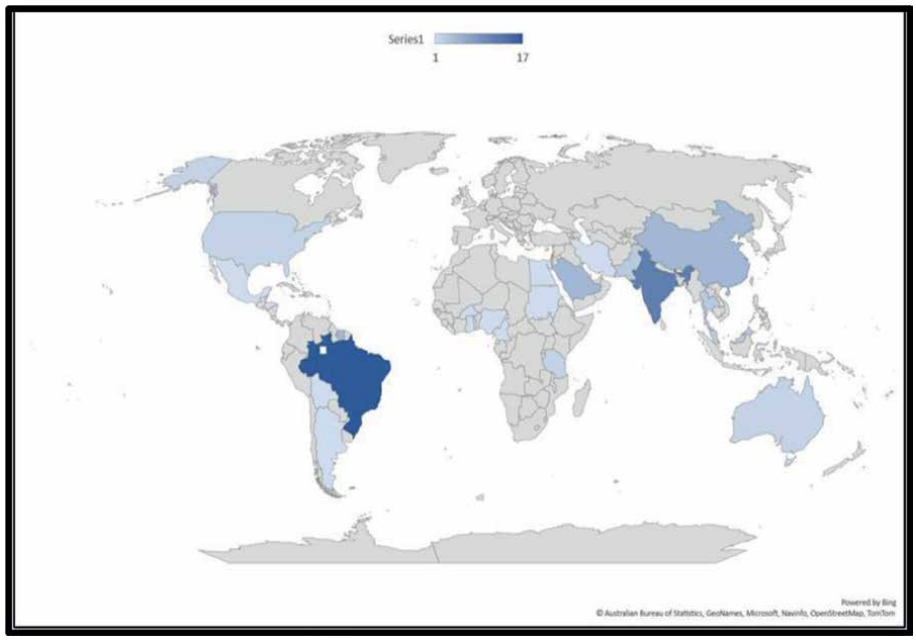

Figure 1.
Regions with documented cases of dengue virus among blood donors.

3. Dengue virus and blood safety: one health perspective

Past outbreaks of emerging infectious diseases have highlighted the close relationship between human and animal health and their environment. A broader understanding of health and disease dynamics demands an integrated approach, which can only be achieved through a convergence of humans, domestic animals, wildlife, and the ecosystem through one health magnifying lens [40–43]. Many include the extinction of some animal species, environmental degradation, environmental pollution, jumping microbial species, and global warming. These are examples of natural drivers of nature that have positively or negatively impacted life on planet Earth and its ecosystems. The emergence and reemergence of infectious diseases endanger not only humans and their livelihood, but other biospheres that support life on earth [44]. Holistic care for our environment and the future of our health has a close connection with what happens in the ecosystem we live in. To safeguard the blood supply that supports the general health of human beings, an interdisciplinary and multi-sectoral approach is paramount. In all measures to protect blood recipients through surveillance systems, disease monitoring, vector control, and environmental conservation, no effort is directed toward an integrated approach through one health system [45].

One Health-One World perception advocates for well-coordinated approaches that will enable a better understanding and management of a complicated health crisis [46, 47]. The only way to solve such problems is to initiate a strategy that integrates all activities in human health, animal health, and environmental preservation into a single system. Different professional leaders and decision-making organs come together collectively to establish an interdisciplinary approach to the treatment of health issues in different communities [48]. Other stakeholders, including global organizations, national governments, and the research community, apply the One-Health approach as a holistic mechanism to combat the spread of infectious diseases. This is one of the best platforms for addressing complex health issues such as emerging and reemerging infectious diseases in blood transfusions [49]. The One-Health and One-World principle focuses on improving our disease surveillance systems in terms of epidemiological trends of diseases and their impact on our economy. It is hoped that one health approach will improve our knowledge of health issues and provide an avenue to develop interventions that are pocket friendly to most counties. A variety of technical, organizational, and sociological factors are an impediment to the long-term implementation of One Health surveillance [50].

In the current world, the world has become a global village and infectious diseases spread rapidly from one nation to another. This is promoted by the interconnectivity of countries through modern transport networks and human movements. This uncomplicated mobility of people around the world shows clearly that no single professional discipline or sector has enough knowledge and resources to prevent the emergence or resurgence of diseases in blood transfusion [51]. The only way to face the future is to eliminate barriers between organizations, individuals, specialities, and sectors. The world requires innovation and collaboration between various sectors to mitigate the frequent threats to human health, livestock health, wildlife, and the integrity of our ecosystem. Current threats and future problems cannot be solved with outdated interventions. The world has become a "One World, One Health" era, and we must develop new mechanisms to handle these threats. Forecasting future threats and working collaboratively in a multidisciplinary approach is the only way to overcome the challenges that arise from emerging infectious diseases in blood transfusions. **Figure 2** is an example of a probable approach through one health that

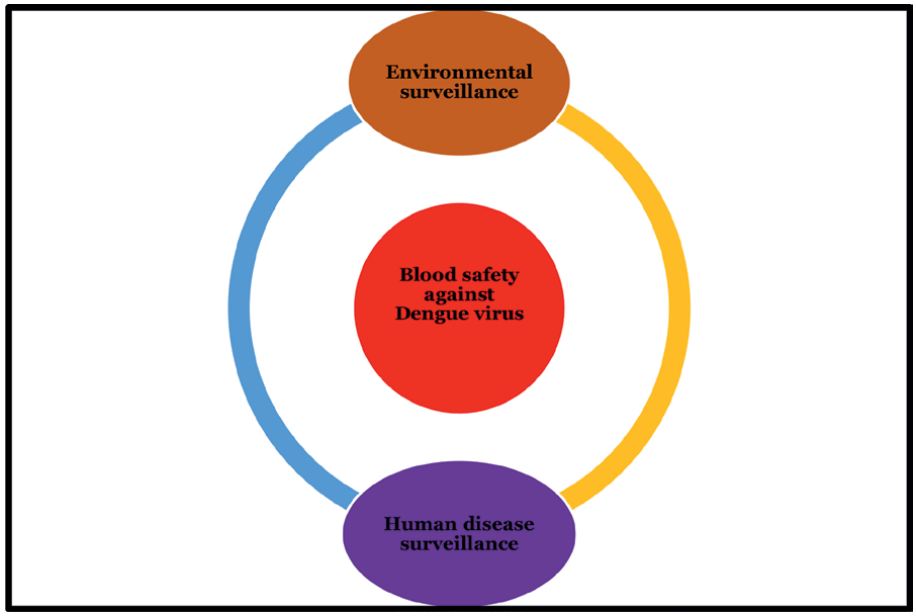

Figure 2.
One health perspective on protecting the blood supply against the dengue virus and other emerging infectious diseases.

will help blood banks keep pace with new threats. A similar approach to address challenges in the fight against diseases in the general human population and in the animal population has yielded good results [52, 53].

3.1 Human disease surveillance

3.1.1 Epidemiological surveillance

A robust surveillance structure is critical for disease prevention for any country in an era full of emerging disease outbreaks. Communication of early warning signs through one health surveillance system helps in preparedness for blood transfusion services. Traditionally, surveillance system models are structured at three levels: event-based, active, and passive surveillance systems [54]. All three systems are applied based on the prevailing circumstances, but with various limitations. One health is the best link to connect all three to address emerging infectious diseases in blood transfusion. Identification of new threats and the ability to share information on risks from passive surveillance would help secure our blood supplies [55]. However, for diagnostic and clinical laboratories, all three surveillance components will be essential to generate surveillance data on viral, bacterial, and parasitic diseases. The basis of disease surveillance is not to investigate all agents, but to investigate data on disease patterns across the world. From a personal understanding, the three surveillance strategies seem inadequate, but they are better than nothing. When used effectively and integrated into our disease mitigation programs, they help predict future disease outbreaks [56].

An active surveillance system is one of the most effective methods used in China to monitor the circulation of infectious diseases in the endemic region of the disease

of the disease [57]. Health officials monitor dengue transmission at the local level and can accurately pinpoint the exact pattern of the disease in their locality. These generate data on the serotypes of viruses that are circulating, the burden of the disease, and the complications associated with dengue infection at any time. The strategy here is to have dengue virus surveillance integrated into routine diagnostic laboratory operation [58]. When properly managed, disease surveillance systems can predict and provide early warning before disease outbreaks. However, due to resource constraints, this is not achievable in low-middle-income countries. Additional active surveillance integrated into BTS will increase the overall cost of blood transfusions [59, 60].

With this approach, disease epidemics can be easily predicted and necessary measures are taken to combat the situation. This initiative-taking surveillance system must have at least two elements focusing on the epidemic or interepidemic period. A sentinel site/physician collaboration, a fever vigilant structure that uses peripheral health workers, and a sentinel hospital system are some types of active surveillance systems [61–63]. The main objective of this surveillance mechanism is to assess and detect disease patterns before there is a rise during the interepidemic period. However, once an epidemic occurs, the focus should be on reducing the spread of infectious diseases. Surveillance strategies must be redesigned and directed toward a contextualized region [64].

Countries with evidence of the presence of dengue virus should have disease surveillance mechanisms as part of their disease prevention intervention. They should also be required to develop a legal framework to recognize dengue as a reportable infectious disease [65–68]. The best place to have this approach would be within the BTS, provided that there are standardized case definitions and a formalized mandated reporting system. Although passive systems are less accurate in prediction and have low specificity because cases are not laboratory confirmed, this can be improved by having them integrated into blood transfusion services. Blood transfusion centers are strategically located and may serve a useful purpose in monitoring and monitoring dengue circulation within a community [69].

The clinical continuum of dengue virus infection illnesses ranges from asymptomatic to the most severe form of DHF/DSS. Clinically, it is usually a difficult task to differentiate between fevers associated with DF and other infectious diseases. As a result, laboratory diagnosis should supplement surveillance. Reporting of dengue disease is best when clinical diagnosis, epidemiological data, and laboratory confirmation are combined [70, 71].

Case reports should be requested from all hospitals, clinics, private physician offices, and other facilities that treat the susceptible population as part of passive surveillance [72]. Because not all clinical cases are accurately detected during low transmission, passive surveillance is insensitive even when required by law [73, 74]. Several individuals who suffer from a mild, non-specific viral condition self-medicate at home without consulting a doctor. Under a passive surveillance approach, considerable transmission has already occurred and may have reached its peak by the time doctors identify and record dengue cases [75].

However, passive monitoring for DF/DHF has two drawbacks. The reporting criteria are uneven, to begin with. Although some nations only report DHF, others also report DF. Second, when reporting instances, the CASE definitions are not always followed. These problems lead to under- and over-reporting, making monitoring systems less effective [76].

Last but not least, the purpose of event-based monitoring is to investigate a strange health occurrence, including fevers with unexplained causes and clustering

of cases [77]. Unlike the conventional surveillance system, event-based surveillance should be an investigation carried out by an epidemiological unit with the support of a microbiologist, an entomologist and other personnel pertinent to the particular event. This will allow the implementation of interventions to stop the further spread of the infection [78].

3.2 Environmental surveillance

3.2.1 Entomological surveillance

To prioritize areas and seasons for mosquito control, it is essential to carry out regular surveillance of Aedes aegypti to identify its distribution, population density, major larval habitats, spatial and temporal risk factors related to dengue transmission and susceptibility or insecticide resistance levels [79]. This information will make it possible to choose and use the most effective mosquito control methods while also keeping track of their effectiveness. Adult and larval populations can be found and tracked using a variety of techniques. Based on monitoring goals, infestation levels, and resource availability, the best techniques [80] are selected. Information about such activities will be of help if shared with blood transfusion services. A risk mitigation strategy that is helpful for transfusion services by providing adequate time to make risk-based decisions.

The Breateau index is the most insightful indicator that shows a connection between homes and positive containers but does not account for container productivity [81]. However, it is desirable to profile larval habitat characteristics while collecting basic information for the Breateau index by simultaneously logging the relative abundance of the different container types, either as potential or actual mosquito production sites, for example, the number of positive drums per 100 houses, number of positive tires per 100 houses [82]. These facts are crucial for concentrating efforts on managing or eliminating the most typical habitats. The rate at which newly emerging adults from different container types contribute to the adult mosquito population can vary significantly. Counting all pupa in each container allows one to estimate the relative adult production [83].

To assess the relative significance of larval habitats, the Pupal index can be broken down into "useful", "nonessential", and "natural" containers or by particular habitat types such as tires, flower vases, drums, and clay pots [84, 85]. This method may not be used for routine monitoring or in every Aedes aegypti population survey because of the practical challenges and labor-intensive efforts needed to achieve pupal counts, especially from large containers. Instead, it can be saved for special studies or used twice in each locality, once during the wet season and once during the dry season, to identify the most productive containers. For practical purposes, the Pupal index has been the most widely used strategy [84, 86]. The basis for making the greatest use of a few resources can be laid by identifying the classes of containers in the neighborhood that have the highest rates of adult emergence. These classes can then be selectively targeted for source reduction or other mosquito control treatments [87, 88]. The pupal/demographic survey is a technique to determine the most important epidemiological container types. Unlike conventional indices previously discussed, pupal/demographic surveys count all pupae in various types of containers in each community [89, 90].

In real practice, a pupal/demographic survey comprises going to a selection of randomly chosen homes. The number of occupants in the house is noted. With the homeowner's consent, the field employees search for the contents of each water-filled

container at each place, strain the contents through a sieve, and then resuspend the sieved contents in a small amount of clean water in a white enamel or plastic pan. Put every pupa in a vial with a label. Large containers provide a great challenge in pupal/demographic surveys, as it is difficult to identify the precise number of pupae in them [86, 90, 91].

Sweep-net techniques with calibration factors have been devised in such circumstances to estimate the overall quantity of pupae in particular types of containers. When returning to the lab, the contents of each vial are moved to tiny cups and covered with mosquito nets fastened with a rubber band if there are any other species except Aedes aegypti in the region. They are maintained until adult emergence, when taxonomic identification and counting can be performed [92]. The collection of demographic data allows us to calculate the ratio of pupae, as reported by Ha and León [85] (a proxy for adult mosquitoes) to people in the community. There is increasing evidence that, when combined with other epidemiological parameters, such as seroconversion rates and temperature specific to dengue serotypes, it is possible to determine the level of mosquito control required in a specific location to prevent virus transmission. This is still an important area of research that needs to be validated. Procedures for sampling adult mosquitoes can provide valuable information for studies on seasonal population patterns, transmission dynamics, transmission risk, and evaluation of adulticide interventions [93].

Planning and evaluating control measures requires knowledge of the sensitivity of Aedes aegypti pesticides. The status of resistance in a population must be carefully monitored in several representative sentinel sites based on the history of insecticide usage and eco-geographical situations to ensure that timely and appropriate decisions on matters like the use of alternative insecticides or the change of control strategies are made [94, 95]. Over the past 40 years, chemicals have been routinely used to prevent mosquitoes and other insects from dispersing illnesses that are crucial to public health. DDT, temephos, malathion, fenthion, permethrin, propoxur, and fenitrothion are only a few of the insecticides widely used that Aedes aegypti and other dengue mosquitoes have become resistant to. The operational influence of resistance on dengue control has not yet been extensively evaluated [96–98]. In countries where DDT resistance has been pervasive, pyrethroid compound precipitated resistance, which is increasingly employed for space spray, is a problem. The voltage-gated sodium channel and mutations in the Kdr gene have been related to resistance to DDT and pyrethroid insecticides in Aedes aegypti because both types of pesticides act at the same target location [99]. Therefore, it is recommended to obtain baseline information on insecticide susceptibility before starting insecticide control operations and to check the susceptibility levels of mosquito larvae or adults [100]. WHO kits to assess the susceptibility of adults and larvae mosquitoes continue to be the accepted approach to assess the susceptibility of Aedes populations. Techniques for analyzing an individual mosquito's biochemistry and immune system have also been created and are currently being used in the field [101].

Integrated community-oriented pest control solutions need the routine monitoring of additional metrics to assess elements such as the number and spread of mosquitoes. These include things such as population density and distribution, settlement traits, land tenure situations, dwelling types, and educational attainment [98, 102, 103]. The planning and evaluation of dengue risk must monitor these characteristics. It is also crucial to understand how home water storage and solid waste disposal techniques have changed over time, as well as how water supply services are distributed, their quality, and their dependability.

Weather data are also crucial in monitoring dengue activities within endemic regions. This information helps to structure epidemic intervention strategies and the planning of focused source reduction and management operations [104]. Some of these data sets are produced by the healthcare industry, so it may be necessary to use additional data sources. For program management, annual or even less frequent updates are generally sufficient. If meteorological data, in particular rainfall patterns, humidity, and temperature, are to be predictive in identifying seasonal trends in mosquito populations and their short-term changes, a more frequent study is necessary [105, 106].

3.2.2 Why one health approach?

One health intervention is a system-thinking approach that helps low- and middle-income countries address threats from dengue and emerging viral diseases in blood transfusion. With limited resources to support their healthcare systems, affected countries will allocate financial resources appropriately where they are needed most. Proper allocation of resources within the different sectors would help most countries deal with collective threats from the dengue virus to blood safety. The avenues available are the establishment of a common laboratory testing facility and an information sharing platform for all sectors involved in dengue surveillance in endemic regions [107, 108].

One health program is an asset to struggling countries that will help them use resources properly to safeguard their blood supply. The challenges facing most blood banks around the world are the lack of adequate financial resources and technology to conduct additional testing of their testing algorithms. The only way to properly use resources is to integrate a system-thinking approach through one health. This approach has had a positive impact on other interventions where one health was implemented to address disease surveillance [109].

Dealing with a complex health problem is a big investment that is not sustainable if only one sector approaches it. Different sectors working collaboratively to address threats to blood safety from emerging infectious diseases provide a sustainable intervention. One Health offers a platform through which different players in blood safety can work with a common goal in mind [110].

Improvement and well-coordination of health systems through one health system is easier than in different sectors working separately. Having a common well-coordinated approach is more impactful and easier to monitor compared to having different players working separately [111–113]. A well-coordinated communication channel between blood transfusion services and other sectors involved in disease surveillance will ease the threats of emerging infectious diseases.

4. Conclusions

In summary, emerging infectious diseases such as the dengue virus threaten the safety of blood transfusions in endemic regions. Necessary measures are required to protect blood recipients from emerging infectious diseases. One Health provides a platform through which various stakeholders, working collaboratively, can ensure that information is available on disease trends in a particular geographic region. Integrating one health into the main disease surveillance system will save most countries millions of dollars in terms of preparation within the blood transfusion sector.

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Conflict of interest

The three authors declare that they have no conflict of interest.

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Section 2

Environmental Aspects

Chapter 6

Bridging Vectors of Dengue Fever: The Endless Cycle

Christopher Mfum Owusu-Asenso

Abstract

Within the past 10 years, there has been a resurgence of arboviral disease outbreaks within the sub-Saharan region of Africa due to the geographic expansion of both the mosquito vectors and their resistance to insecticides. The reasons for this resurgence are not well understood, migration of people, movement of disease vectors, and deforestation as a result of rapid and unplanned urbanization may lead to increased erosion of their natural habitats leading to contact with humans, and/or previously obligate sylvatic species might acclimatize to new urban environments and hosts, potentially with a greater role as vectors. And lack of effective control methods for *Aedes* mosquitoes. The possibility of arboviruses to adapt to new vectors rapidly occur, and this can have great significant consequences. Other *Aedes* species such as *Aedes africanus* and *Ae*. *luteocephalus*. play a vital role in the transmission of arboviruses in Africa because they are involved in sylvatic arbovirus transmission cycles and can also act as a bridge vector to humans. Bridge vectors may initiate a human outbreak, but large epidemics typically occur only when virus transmission involves urban populations of *Ae*. *aegypti* or *Ae. albopictus*, which has the ability to feed on both humans and other vertebrates.

Keywords: Dengue fever, one health, *Aedes* mosquitoes, bridging vectors

1. Introduction

The Aedes mosquito is a significant carrier of arboviruses, including the Zika virus, dengue virus, chikungunya virus, and yellow fever virus [1]. *Aedes aegypti* originated in Africa, spread to other continents through trade and travel, and is now distributed worldwide. These vectors have accelerated the urban spread of these viruses in both tropical and temperate climates, **Figure 1** [2]. Tropical urbanization and the extremely effective and anthropophilic *Aedes aegypti's* colonization of their increasing habitat pose the biggest health danger from arboviral disease emergence [3].

Despite extensive attempts to help contain or eradicate their outbreaks, the majority of arboviral diseases continue to be more prevalent in Africa for a variety of reasons [4, 5]. Arboviral illnesses are not exempt from concerns about public health. Recent epidemics of arboviral infections in numerous countries have enhanced the significance of Aedes vectors in sub-Saharan Africa. Due to their

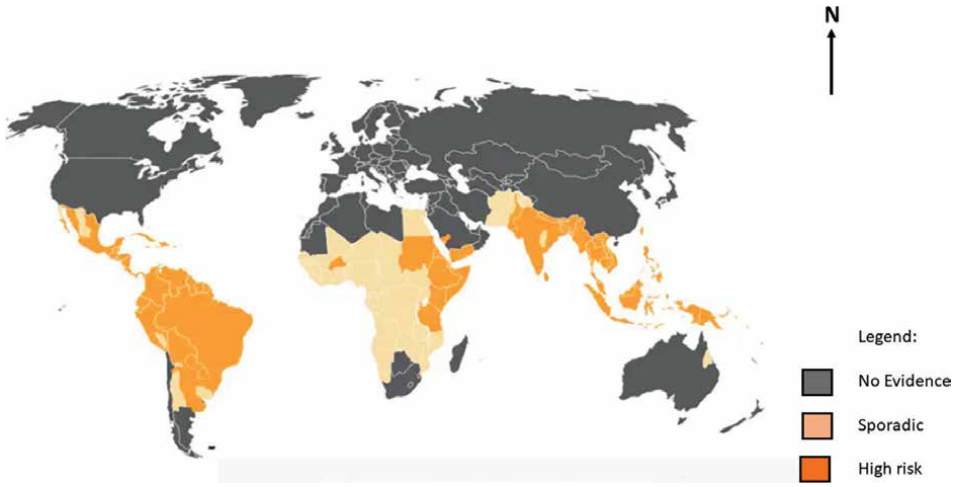

Figure 1.
A world map showing risk transmission of Dengue fever.

associations with human arboviral infections like Zika, dengue, chikungunya, and Yellow fever, *Aedes* aegypti and *Aedes albopictus* have received significant attention [4, 6]. The vectors have been implicated in most epidemics within sub-Saharan Africa [7]. In the past five years, dengue epidemics have occurred in Burkina Faso in West Africa. Faso [8, 9] Cote d'Ivoire [10, 11], Senegal [12], yellow fever in Cote d'Ivoire [13], and Nigeria [14]. The recent confirmation of dengue cases has occurred in Ghana [15–17].

In the African continent, arboviral diseases have become a major public health threat [18]. The re-emergence of arboviruses such as the Dengue virus, and Chikungunya virus, is associated with urbanization, trade, and travel [3]. With 10–20 million cases reported annually in Africa. Furthermore, about 250,000–500,000 cases of Dengue hemorrhagic fever, 20,000 fatalities, and 264 disability-adjusted life years per million people each year have been documented [19]. No single intervention will be enough to control arboviral diseases, according to research and arboviral control experts, regardless of the effectiveness of future initiatives [20].

Currently, there are no effective vaccines or treatments for several important human-infecting arboviruses including the Dengue virus and Zika virus [21]. Therefore, the control of mosquito vectors is still the main tool to eradicate, or at least reduce, the incidence of arboviral diseases. This vector control relies heavily on the use of insecticides, the effectiveness of which may be impacted by resistance. The emergence of resistance of vectors to the four major classes of insecticides (i.e., organochlorides (OCs), pyrethroids (PYs), carbamates (CAs), and organophosphate (OPs) are highly widespread [4]. This has reached an extensive level geographically and across vector species [22–24].

Several newly emerging arthropod-borne viruses (arboviruses), including dengue, yellow fever, chikungunya, and zika viruses, are a result of sylvatic transmission cycles, in which bridging Aedes mosquitoes spread the viruses among non-human primates. A crucial, but poorly understood phase in the formation of arboviruses is the initial virus overflow from the sylvatic cycle to the human population. This review discusses bridging vectors of arboviral diseases from the standpoint of a One-Health control strategy.

2. *Aedes* as vectors of arboviruses

Aedes is a genus of mosquitoes originating from the tropical and subtropical regions [25]. However, these vectors are now distributed on all continents except for Antarctica. The visible black and white markings on their body and legs are distinctive of *Aedes* mosquitoes. These vectors are diurnal, with peak biting periods early in the morning and in the evening before dusk [26].

Some species of the *Aedes* genus are well-known for various arboviral diseases, but the most prominent species that transmit arboviruses leading to epidemics are *Aedes aegypti* and the highly invasive *Aedes albopictus* [6].

2.1 *Aedes aegypti*

The *Ae. aegypti* can be identified by white markings on its legs and a marking in the form of a lyre on the superior surface of its thorax, **Figure 2**. This mosquito originated in Africa [27].

Only the female bites for blood, which is essential to induce egg laying and for maturing and nourishing her eggs. To find a host, these mosquitoes are attracted to chemical compounds (cues) emitted by mammals, including ammonia, body temperature (heat), carbon dioxide from sweat and breathing, lactic acid from certain bacteria, octanol from sweat, cholesterol, folic acid, skin lotions, and perfume [28]. Adults of the *Ae. aegypti* are highly domesticated mosquitoes and highly anthropophilic [29], and typically endophilic. Although *Aedes aegypti* mosquitoes most commonly feed at dusk and dawn, in shady areas, or when the weather is cloudy, they can bite and spread infection all year long and at any time of the day [28, 29]. The *Aedes aegypti* is more closely associated with human habitation The larvae develop preferentially in artificial containers [30, 31], including discarded car tires, toilet tanks, and water storage vessels often in urban settings. Although the lifespan of an adult *Ae. aegypti* is two to four weeks depending on environmental conditions [32], the eggs can be viable for over a year in a dry state, which allows the mosquito to re-emerge after hibernation or aestivation. The anthropophagic behavior of the *Ae. aegypti* is dependent on the expression of the odorant receptor AeegOr4 [33].

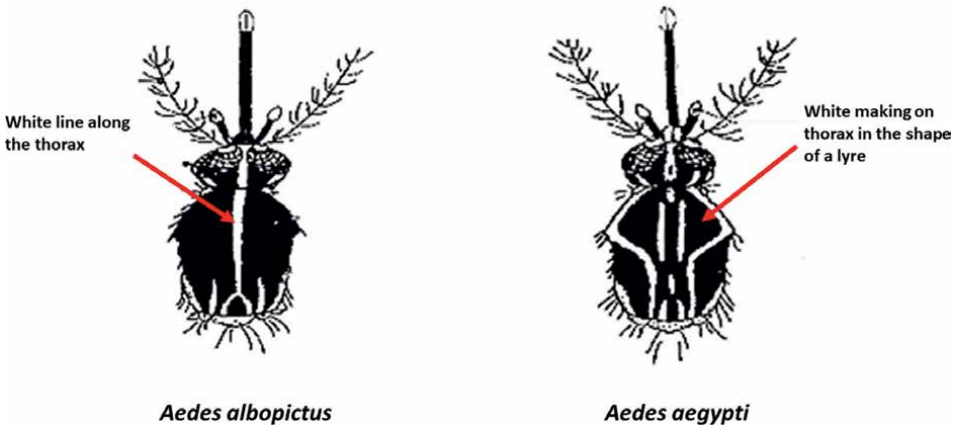

Figure 2.
A pictorial morphological identification of Aedes aegypti and Ae. albopictus.

Ae. aegypti breeds in both sylvatic and domestic environments in artificial containers within or in proximity to human habitation whereas larvae of the sylvatic ecotype are bred in natural habitats such as rock pools, tree holes, plant axils, and fruit husks [31]. Larvae of the two *Ae. aegypti* ecotypes are exposed to different bacterial groups in their respective breeding sites, possibly resulting in variances in vectorial capacity [34]. Naturally, two morphological subspecies have been identified that generally inhabit these ecotypes: *Ae. aegypti aegypti* and *Ae. aegypti formosus*. Evidence however shows that, *Ae. aegypti formosus* is increasingly found in urban environments [31], and the indicative morphological characteristics i.e. presence/absence of white abdominal scaling patterns [35] often differentiate the variety. On the contrary, clear genetic boundaries are absent, probably as a result of widespread current or recent historical gene flow [36, 37].

2.2 *Aedes albopictus*

Aedes albopictus, denoted as the Asian tiger mosquito, the most invasive species of the Aedes genus, occurs even in temperate regions **Figure 2**. In recent times the distribution of *Ae. albopictus* from Asia to Africa, Europe, and the Americas through the used tire trade has heightened [38]. *Aedes albopictus* in contrast to *Ae. aegypti* is usually exophagic and bites humans and animals opportunistically [36], but it has also been shown to exhibit anthropophilic behavior similar to *Ae. aegypti* [2, 36]. *Aedes albopictus* thrives in a diverse range of breeding habitats than *Ae. Aegypti*. They also show comparable larval development behavior in artificial containers such as *Ae. aegypti*. This diversity of habitats of *Ae. albopictus* explains its abundance in rural as well as peri-urban areas and shady city parks, feeding readily on a diversity of mammalian and avian species [39].

2.3 Other *Aedes* species

The possibility of arboviruses adapting to new vectors rapidly occurs, and this can have great significant consequences [4]. Other *Aedes* species play a pivotal role in the transmission of arboviruses in Africa because they serve as a link between the sylvatic and human transmission cycles and/or are involved in sylvatic arbovirus transmission cycles. *Aedes africanus* is considered the main vector of yellow fever virus in Africa within the sylvatic environment [4] and can also act as a bridge vector to humans, together with *Ae. luteocephalus, Ae. taylori, Ae. bromeliae*, *Ae. furcifer, Ae. metallicus, Ae. opok, Ae. vittatus*, and species of the *Ae. simpsoni* complex [40]. Sylvatic dengue viruses in Africa are transmitted among non-human primates by *Ae. furcifer* and *Ae. luteocephalus* within the sylvatic habitat, and usually cross over to humans through biting by *Ae. furcifer* [40]. Bridge vectors may initiate a human outbreak, but large epidemics typically occur only when virus transmission involves urban populations of *Ae. aegypti* or *Ae. albopictus*, though there can be exclusions. The mainstream of these *Aedes* vector species are established in rural or forest areas, and so, are less likely to present a threat in the urban environments where *Ae. aegypti* populations thrive. Nevertheless, increasing erosion of their natural breeding habitats could lead to human-vector contact, and/or previously obligate sylvatic species might acclimatize to new urban environments and hosts, potentially with a greater role as vectors [3]. Many readily feed on animals both domestic and wild non-human

primates, as well as humans, hence their potential importance as bridging vectors and zoonotic transmissions [41].

3. Dengue fever: a zoonotic disease

Many zoonotic diseases are caused by various contacts and frequently intricate cycles of transmission between people and animals, both vertebrates and invertebrates, as well as evolving social and environmental factors. Prior research has demonstrated that environmental, animal, and human factors all contribute to imported dengue cases and cyclical epidemics, which pose a threat to public health, **Figure 3** [42].

Continuous human land use in biome ecotopes for habitation, agriculture, or livestock increases the risk of spillover occurrences and the transmission of zoonotic diseases [43].

Public health institutions should ideally be structured around principles such as; integration, personnel empowerment (favoring prompt decision-making by health agents on the ground), community engagement by educating communities about best practices and bolstering control efforts, and flexibility to assign health agents in accordance with the current emerging or seasonal public health treat [44]. Tropical illness monitoring, however, is segmented and autonomous from one another in the majority of Sub-Saharan African countries.

Adopting a One Health approach enables the inclusion of more interconnected factors, such as the environment, land use, and management (such as the disposal of plastic containers, methods of water storage due to the availability of piped water, etc.), as well as social and climatic factors that affect disease transmission patterns. Due to their high occurrence rates, vector-borne diseases are of the utmost importance for public health. Among these, the dengue virus (DENV), which is spread by *Aedes* mosquitoes, causes disease with a high global morbidity and fatality rate.

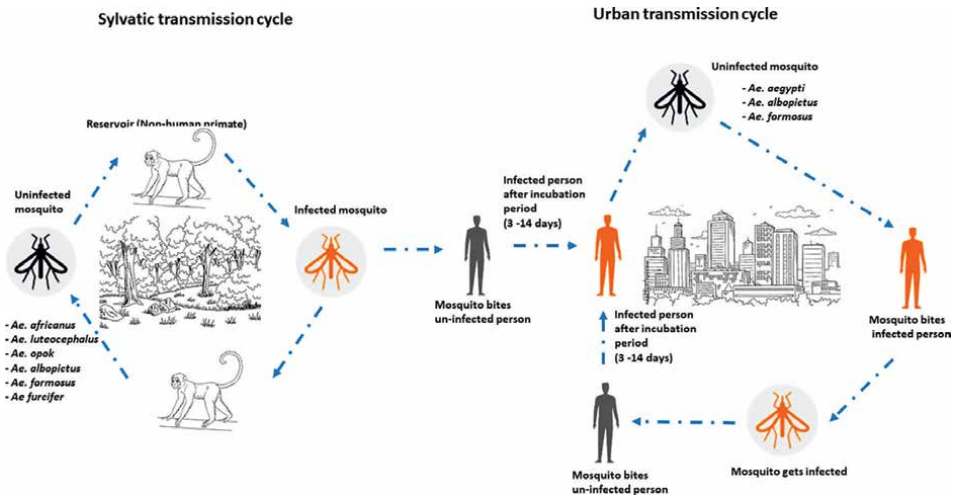

Figure 3.
Transmission cycles of Dengue fever.

4. Urbanization: a cause of arboviral disease spillage

According to [45], urban landscapes affect the spatial variability of mosquito abundance, community structure, mosquito-host interactions, and infection rates. *Aedes* mosquitoes are regarded as important vectors for public health due to their vector competence, proximity to, and ability to feed on human blood. Therefore, reducing the risk of human arbovirus infection requires a better knowledge of how urban settings affect mosquito numbers, blood-feeding behavior, and infection status in Aedes mosquitoes.

5. Insecticide resistance in *Aedes* mosquitoes

One of the effective approaches to swiftly interject the transmission of arboviruses is to employ safe and effective insecticides against mosquito vector populations that include both adults and larvae [20]. While insecticide-based interventions have effectively reduced *Aedes* mosquito populations for many years, resistance has recently emerged due to the reliance on a few active components approved for use in public health [46]. An insecticide's effectiveness or level of control may be reduced due to insecticide resistance (IR), which is a shift in the mosquito population's susceptibility to the substance. Insecticide resistance has emerged in *Ae. aegypti* in all four classes.

There is a dearth of information on pesticide resistance in Aedes mosquitoes worldwide, with the majority of the reports received from South-East Asia and Latin America [46]. It has been reported from every region where DDT has been tested that both *Ae. aegypti* and *Ae. albopictus* have developed widespread resistance to the insecticide [47]. There have been proven reports of Ae. aegypti from the regions of West, Central, and East Africa indicating patchy resistance to pyrethroids (mainly permethrin and deltamethrin) [46]. However, it should be highlighted that because Ae. aegypti discriminant doses are lower, pyrethroid doses are used for An. gambiae are frequently used to analyze pyrethroids in Aedes mosquitoes, which may lead to an underestimation of resistance,

In Yaoundé, Cameroon, recent bendiocarb testing on both *Ae. albopictus* and *Ae. aegypti* revealed resistance [48]. Fortunately, the first-line biological and chemical larvicides, Bti and temephos, have not recorded any resistance.

Bacillus thuringiensis israelensis's (Bti) complex method of toxicity and the lack of any recent reports of resistance in Aedes field populations predict susceptibility. Temophos resistance is highly prevalent in Asia and Latin America [46]. However, due to Africa's reportedly complete susceptibility, temephos is viewed as a potential option for water treatment. In contrast to *Ae. aegypti*, resistance in *Ae. albopictus* seems to be relatively low [4]. This could be because *Ae. aegypti* mosquitoes have had more prior exposure to indoor spray treatments and home insecticides than *Ae. albopictus* mosquitoes. Insecticide resistance will almost certainly eventually have a detrimental impact on our ability to control this vector shortly due to the expansion of *Ae. albopictus* populations into areas with high insecticide use; adulticides, or selection pressure from agriculture in its new breeding sites [47].

There are a variety of potential adaptations that allow a mosquito to endure dangerous levels of an insecticide; these adaptations are typically categorized based on their biochemical/physiological features as either mechanism of lower exposure i.e. increased excretion or reduced absorption and detoxification, or mechanisms of

decreased reactivity to the insecticides (changes in the target site) [49]. Most of the time, the insecticide is either detoxified or sequestered before it reaches its target site due to variances in detoxifying enzymes or changes in the sensitivity of the insecticide target caused by mutations, which reduce the insecticide's affinity for its target [50].

6. *Aedes* vector control strategies: a One-Health perspective

To gather epidemiological data for use in informing decisions and taking action, participatory rural evaluation methods are applied in entomological surveillance and disease monitoring. The epidemiological situation, spatiotemporal distribution, and risk of disease transmission are significantly improved as a result of this method [51]. Understanding host-pathogen-environment relationships, developing tools and technologies, modifying people's behavior, and assessing the efficacy of interventions are all part of entomological surveillance and disease monitoring. Interest in multi-sectoral, socioeconomic, systems-based, collaborative (MSC) study techniques such as One Health is spurred by the need to adequately forecast, prevent, and respond to infectious diseases that emerge unexpectedly from human-animal-environmental systems. MSC research, which can be categorized as a form of "pragmatic research," may be particularly helpful in documenting changes in complex human-animal-environmental systems, expediting the research-to-action process, and assessing the efficacy of interventions [52].

Using the frameworks of adaptive management and one-health, a plan will be created to identify, collect, and share linkages between important elements of regional complex systems of arboviral disease. Based on currently available scientific knowledge and input from stakeholders, significant causal relationships between social, economic, and environmental factors that are a determinant of arboviral disease could be identified at different levels, and assumptions that guide interventions may be offered. Implementing a One Health strategy thoughtfully and comprehensively can be difficult, especially in the face of a perceived crisis.

7. Conclusions

Vector control for *Aedes* mosquitoes is one of the main strategies against arboviral disease transmission, but it is mostly insecticide-based, which induces resistance in mosquitoes and also may target non-target species and cause damage to the environment. This resistance is probably due to the lack of regulation in use and the dosage of each case. Dengue fever control and prevention around the world should implement the One-Health approach. Furthermore, a global strategy and a global framework for Dengue fever control will be suitable for one health strategy which uses a multi-disciplinary sector for this effort. One-Health approach will manage the strategy of the health workforce in multidisciplinary and other communities to provide health services and collaborate to control all factors involved in the transmission of DF, such as human health, animal health, environmental, socioeconomic, politics, and other sectors related. This review supports the need to generate mosquito control strategies using a One-Health approach for sustainable and effective vector control of the Dengue vector.

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Conflict of interest

The author declares there is no conflict of interest

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Section 3

Pathogenicity

Chapter 7

Dengue Virus Encephalitis

Wesley Gabriel Novaes Botelho, Alexander Daronco, Maiara Aline Daga and Alcântara Ramos de Assis César

Abstract

In this chapter, we will draw attention to the possibility of viral encephalitis caused by a common pathogen: dengue. To this end, it includes necessary knowledge of diagnosis and therapeutic management, such as general notions of infections in the central nervous system; viral encephalitis—diagnostic and therapeutic investigation; arboviruses and their relevance; dengue as an endemic disease; diagnostic methods and treatment; its possible clinical presentations and complications, among them, dengue encephalitis.

Keywords: encephalitis, viral, dengue, epilepsy

1. Introduction

In this chapter, the authors focus their efforts primarily on understanding encephalitis, especially those caused by viral pathogens, as well as their neurological repercussions. It brings fundamental concepts for understanding diagnostic reasoning and prognosis. Besides the various viruses already well known and with possible and consecrated research and therapeutics, it also intends to draw attention to less prevalent etiologies. However, it is of fundamental importance for the medical community and is of paramount importance for neurology.

Among the pathogens, the chapter draws attention mainly to dengue virus encephalitis, due to the high incidence of dengue cases in endemic countries and encephalitis being a rare consequence but difficult to diagnose and extremely important for medical knowledge in the scope of diagnosis differences in countries where dengue is endemic.

2. Central nervous system infections

Infections of the central nervous system (CNS) present a wide variety of situations, ranging from common diseases to serious and rare diseases, from benign manifestations to severe neurological impairments, which often determine sequelae and cause the patient's death, in addition to acute, subacute, and chronic diseases.

The main clinical manifestations of infections are characterized by headache, fever, and altered mental status. Vomiting and focal signs may occur, but even so, these symptoms are common to several other neurological diseases that mimic

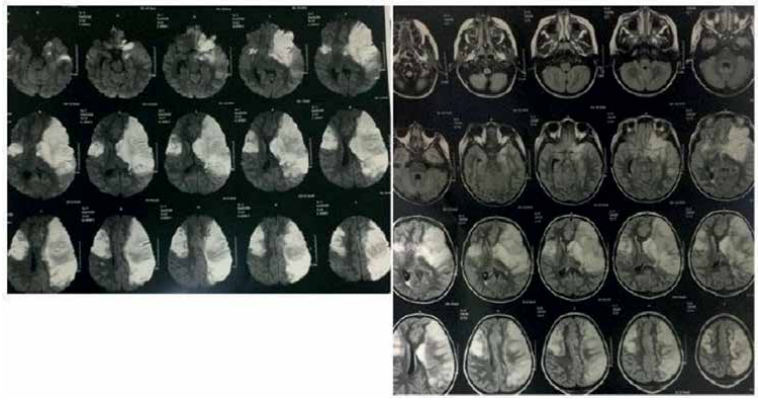

Figure 1.
Patient with stroke after acute dengue infection. Right: Brain MRI in diffusion sequence. Left: Brain MRI in FLAIR sequence. Both show the presence of an extensive lesion with diffusion restriction and hypersignal on the images, respectively, in the territory of the middle cerebral artery, with involvement of the ipsilateral thalamus and mass effect with midline shift. In addition, the presence of a right temporal parietal lesion is observed. Extracted from: http://www.rmmg.org/article/details/2631.

meningitis and encephalitis. For the diagnosis, a complete anamnesis, epidemiological history, and accurate clinical and neurological examinations are essential to glimpse signs that may suggest a probable diagnosis (**Figure 1**) [1].

3. Viral encephalitis

Encephalitis is the term used to describe the involvement of the brain tissue by a viral etiological agent, while bacterial, fungal, or focal parasitic infections involving the brain tissue are classified as cerebritis or abscess, depending on the presence or absence of a capsule [2]. It is characterized by the presence of an inflammatory process in the brain parenchyma, associated with clinical evidence of brain dysfunction. This process is often caused by a viral infection. The main viruses that cause encephalitis in immunocompetent patients belong to the group of herpes viruses, arboviruses, and enteroviruses [1].

Although, by definition, in cases of meningitis, the infectious/inflammatory process is limited to the meninges, in encephalitis, not rarely, the process is not restricted to the brain parenchyma, and the involvement of the meninges can also be present [2].

Some authors consider that up to 2/3rd of encephalitis cases will not have an etiological definition [3]. Among cases with identified etiologies, viruses represent the largest share of encephalitis cases. The true incidence of these infections is difficult to determine, as a diagnosis may not be considered at first or a specific viral etiology may not be confirmed due to the lack of laboratory structure for this [4].

Several viruses can penetrate the central nervous system, causing, among other conditions, encephalitis. Herpes Simplex Virus (HSV1) is a relatively common cause of encephalitis, which together with Varicella Zoster are described in the medical literature as classic etiologies [5]. Other viral pathogens may be suggested by local epidemiology, as is the case with the dengue virus. Acute arbovirus infection occurs 5 to 15 days after the bite of the transmitting mosquito. CNS invasion can occur during the initial phase of viremia with infection of capillary endothelial cells and subsequent infection of neurons. The viral infection spreads from one neuron to another

through dendrites and axons, predominantly affecting the gray matter of the cerebral cortex and the basal ganglia [1].

Viral encephalitis can also be classified, according to the stage of infection, into viral and post-viral. In direct viral infection, it is possible to find genetic material or the virus itself in the histology of the affected brain tissue. In postinfectious encephalitis, the virus does not directly attack neurons, but the intense immune-mediated inflammatory response can lead to demyelination and neuronal damage [6].

3.1 Clinical presentation

Viral encephalitis should be suspected in the presence of a febrile illness accompanied by headache, altered level of consciousness, and also signs and symptoms of brain dysfunction, such as: cognitive dysfunction (memory), disorientation, hallucinations, agitation and changes in behavior, and focal neurological alterations (hemiparesis, aphasia), in addition to epileptic seizures. Some clinical findings may suggest an etiology, such as rash in the case of arboviruses, parotitis (mumps), gastroenteritis (enterovirus), and upper airway infections (influenza and HSV1) [1].

3.2 Diagnosis

After clinical suspicion, a complementary arsenal should be used, such as cerebrospinal fluid (CSF) puncture associated with neuroimaging. The European Federation of Societies of Neurology, EFNS, recommends that CSF investigations include basic chemical and cytological analysis and research of specific viruses using the polymerase chain reaction technique.

Laboratory tests (hemogram, VHS, C-reactive protein), in addition to blood cultures, chest X-rays, and examinations for other possible sources of infection such as clinical evaluation, electroencephalography (EEG), and brain biopsy, should only be indicated if the previous tests are not conclusive [1].

3.3 Radiological presentation of encephalitis

A plethora of infectious and non-infectious inflammatory diseases affect the central nervous system (CNS). The normal brain responds to these injuries in a limited way, often causing injuries to the parenchyma. Initially, there is an increase in cerebral perfusion caused by the release of inflammatory factors and cytokines, later, and as a consequence, the capillaries leak, leading to edema but without pain, unless the meninges are affected. In most cases, there is a concomitant abnormality of the blood-brain barrier with the associated enhancement. Later, if the insult results in neuronal death, the tissue shrinks and becomes atrophic [7].

Imaging techniques are relatively sensitive for detecting an abnormality, localizing it, and, in many cases, categorizing the lesion into infectious/inflammatory *versus* neoplastic or vascular disease. The location of the lesions is the critical first step in the differential diagnosis. Lesions may occur in a variety of locations, such as the epidural, subdural, subarachnoid, intraventricular, or intraparenchymal space; white matter; gray matter; gray-white junction; or deep gray matter. The lesions may be confined to a particular region of the brain, such as the temporal lobe, or they may be scattered, affecting different areas and presenting numerous clinical manifestations. Among the various imaging methods, including magnetic resonance spectroscopy (MRE), perfusion-weighted imaging, and single-photon emission computed

tomography (SPECT) examination, only DWI had an impact on the diagnostic suggestion of infectious/inflammatory conditions, because diffusion restriction is characteristic of some stages of some infections [7].

3.4 Initial conduct

The *European Federation of the Neurological Societies* (EFNS) also recommends performing neuroimaging, preferably MRI, before lumbar puncture in patients with immunosuppression, previous CNS disease, recent seizure, papilledema, altered level of consciousness (Glasgow coma scale <10), or focal neurological (except cranial nerve palsy).

For conceptual reasons, the neuroimaging exam is almost always the first one to be performed. Regardless of whether the lumbar puncture is performed, if there is clinical suspicion of acute viral encephalitis, good practice establishes the initiation of treatment with acyclovir at a dose of 10 mg/kg intravenously (IV) every 8 hours for at least 14 days before a diagnosis is confirmed. Etiology is possible. The rationale for this practice is that acyclovir is a relatively safe treatment and, when administered in herpetic encephalitis before the patient falls into a coma, reduces mortality and morbidity in treated patients. Thus, acyclovir treats the most common and severe viral encephalitis, as well as covers varicella zoster virus (VZV) infection.

All cases of acute viral encephalitis should be managed in an intensive care unit (ICU) with mechanical ventilation available. Regardless of the etiology, clinically supportive therapy is one of the mainstays of the treatment of acute viral encephalitis.

Epileptic seizures should be controlled with IV phenytoin or other drugs that are necessary. Extreme attention should be given to maintaining breathing, heart rhythm, and fluid balance; prevention of deep venous thrombosis and aspiration pneumonia; and clinical control of intracranial hypertension (ICH), and secondary bacterial infections [1].

Secondary neurological complications in the presence of viral encephalitis are frequent and include cerebral infarction, cerebral venous thrombosis, syndrome of inappropriate secretion of antidiuretic hormone, aspiration pneumonia, upper digestive hemorrhage, urinary tract infection, and disseminated intravascular coagulation (DIC). Isolation in acute viral encephalitis is only indicated for patients who are very immunocompromised, with rabies encephalitis or with rashes and contagious hemorrhagic fever.

3.5 Treatment

When the clinical history and thorough general physical and neurological examinations raise the suspicion of acute viral encephalitis without directing to a particular etiology, good practice recommends the empiric initiation of acyclovir for the reasons mentioned above. However, when there are clinical and laboratory data that guide the etiological diagnosis for a given virus, the treatment must be adapted.

In the case of herpetic encephalitis in an adult patient, acyclovir is maintained at a dose of 10 mg/kg IV every 8 hours for at least 14 days. VZV encephalitis can also be treated with this regimen, and when severe, usually associated with encephalic vasculitis, high-dose dexamethasone or pulse therapy with methylprednisolone for 3 to 5 days should be associated. In the case of CMV encephalitis, treatment with ganciclovir at a dose of 5 mg/kg, IV, every 12 hours, associated with foscarnet at a dose of 60 mg/kg, IV, every 8 hours, or 90 mg/kg, EV, every 12 hours, for a period not

Virus	Antivirals	Duration (days)
HSV-1, VZV, HSV-2 *(adults)*	Acyclovir 10 mg/ kg IV every 8 hours	14–21
COGS	Ganciclovir 5 mg/ kg IV every 12 hours + foscarnet 60 mg/ kg IV every 8 hours or 90 mg/ kg IV every 12 hours	indefinite

HSV: herpes simplex virus; VZV: varicella zoster virus; and CMV: cytomegalovirus.

Table 1.
Specific antiviral regimens and mean duration of treatment.

yet determined in studies. If the patient has AIDS, antiretroviral treatment should be initiated or maintained, independing on the case [1].

So far, acute viral encephalitis caused by other viruses has no recommended specific treatment, and the patient must receive intensive clinical support until the natural resolution of the process. A summary of the specific antiviral treatments available is shown in **Table 1**.

4. Arbovirosis

Arboviruses are diseases of viral etiology, caused by Arboviruses, transmitted from infected hematophagous arthropod vectors [8]. Arboviruses (*Arthropod-borne virus*) have part of their replication cycle, especially the initial one, in such insects and can be transmitted to mammals and other animals through bites. It is considered that around 150 different arboviruses cause diseases in humans [9].

After the bite of the infected insect, the dendritic cells of the skin are initially affected. After initial replication, migration to lymph nodes occurs, and the period of viremia begins, with variable duration, which can occur 3–5 days. Arboviruses disseminate to the liver, spleen, and bone marrow and may reach other organs [3].

Such agents can cause a series of diseases with different clinical presentations, ranging from mild and self-limiting forms to severe forms characterized by syndromes such as shock due to plasma leakage and even invasion of the central nervous system, causing encephalitis [10].

4.1 Epidemiology and geographical distribution of arboviruses

Arboviruses are distributed throughout the globe, with the exception of Antarctica, and predominate, above all, in regions of the globe with a tropical and subtropical climate. Such distribution respects the biomes in which the vectors tend to have better adaptation [8].

Until the 1970s, dengue was considered a disease restricted to some countries in the Caribbean and Southeast Asia. From that time on, there was a lack of control of the vector *Aedes aegypti*, with its migration to other countries. Today, it is estimated that 2.5 billion people live in risk areas in approximately 100 countries. About 50 million dengue virus infections occur annually, with 500,000 cases of dengue hemorrhagic fever and 22,000 deaths. There have been two major recent pandemics, in 1998 and between 2001 and 2002, with twice as many cases of dengue hemorrhagic fever reported in the last pandemic than in 1998. The lack of an available vaccine, the presence of four antigenically distinct serotypes, and the lack of specific therapy make combating the mosquito vector the only effective measure to control the disease [11].

Arthropod-borne encephalitis viruses represent a significant public health problem in most tropical and subtropical countries. These viruses belong to the *Flaviviridae*, *Togaviridae*, *Bunyaviridae,* and *Reoviridae families*. Such agents are highly adaptive to specific reservoir hosts and are transmitted from animal to animal through the bite of an infected arthropod [9].

The main viral agents responsible for encephalitis in humans and belonging to the *Flaviviridae family* are distributed heterogeneously across the globe. Dengue viruses DENV-1, DENV-2, DENV-3, and DENV-4 are transmitted by *Aedes aegypti* or *Aedes albopictus mosquitoes.* The worldwide distribution of vectors and different serotypes has increased dramatically over the last decade, and PAHO/WHO data suggest that 3.9 billion people are subject to infection. In 2015, dengue virus infections in Southeast Asia, the Americas, and the Eastern Pacific totaled 3.2 million, and the incidence rate in the Philippines reached 24%. South Asian countries have reported seropositivity for antibodies against the dengue virus ranging from 60 to 80%. In Africa and the eastern Mediterranean, data are scarce due to underreporting; however, the vectors are present in 15 countries. In Europe, transmission is low, with most cases being travelers from endemic countries. In 2020, 5 cases of local transmission were reported in Italy. Regarding the neurological involvement by dengue, about 1% of those infected evolve with this outcome, with brain involvement being predominant, ranging from 58.8 to 71.4% in case report studies [12–14].

Other members of the *Flaviviridae family* are extremely important in cases of encephalitis worldwide. The Japanese encephalitis virus is responsible for 68,000 cases in Asia. Murray Valley encephalitis virus affects countries in Oceania, and its incidence is underestimated, since only 1 in 150–1000 infected people develop the disease. Encephalitis of St. Louis has, in outbreaks, an incidence of 5 to 200 cases per 100,000 people, and in the last 50 years, it has affected more than 10,000 people. West Nile encephalitis virus is widely distributed in Africa, South Asia, the Middle East, and Europe [9].

4.2 Major arboviroses

We can list 4 main families responsible for encephalitis in humans. They are: *Flaviviridae*, *Togaviridae*, *Bunyaviridae,* and *Reoviridae.*

4.2.1 Flaviviridae

The *Flaviviridae family* comprises about 70 species, 40 of which are responsible for diseases in *Homo sapiens*. Classically, the Flavivirus family is divided into 4 main branches: tick encephalitis complex, Japanese encephalitis complex, yellow fever, and dengue. All representatives of the family can be responsible for infections in humans, with possible neurological repercussions [15].

Flaviviruses have particles that vary between 40 and 60 nm and have an icosahedron-shaped capsid covered by a lipid envelope with membrane proteins and glycoprotein spikes. Positive single-stranded RNA makes up its genome. Its replication is cytoplasmic, using host machinery and producing a strand of reverse polarity, used as a template.

The encephalitis virus of St. Louis has a tropism for the nervous system, with direct invasion and neuronal damage. It causes illness with nonspecific symptoms of encephalitis, such as seal signs, fever, and headache. Regarding mortality, data

ranging from 5 to 20% have already been recorded. In Brazil, descriptions of co-infection with dengue have already been registered, and the picture presents with hemorrhagic lesions and positive tourniquet test, with unfavorable outcome.

The West Nile virus is also neurotropic and mainly affects the medulla, pons, thalamus, substantia nigra, and basal ganglia, producing clinical syndromes of the first motor neuron; however, the symptoms may be compatible with other encephalitis. The disease manifests itself more intensely in the elderly and immunosuppressed. The treatment is supportive, intervening in convulsive crises; some case reports predict advantages in the use of corticotherapy and immunoglobulin [15].

Japanese encephalitis is a clinical entity that presents signs and symptoms of non-specific acute encephalitis. The incubation period for the virus lasts 5 to 10 days, and evolution to neuroinvasive disease occurs in a minority of cases. There is no targeted therapy for Japanese encephalitis virus.

4.2.2 Togaviridae

Togaviruses are a family of single-stranded, enveloped, spherical RNA viruses classified into two genera: *Rubivirus* and *Alphavirus.* This includes 40 members, and the genus *Rubivirus* is composed of a single member, rubella. The main encephalitis-related agents are Western equine encephalitis virus, Eastern equine encephalitis virus, Venezuelan equine encephalitis virus, and *Mayaro virus* [15].

4.2.3 Bunayviridae

The four genera belonging to the family *Bunyaviridae* that infect animals are: *Orthobunyavirus, Phlebovirus, Nairovirus, and Hantavirus.* These viruses are transmitted by mosquitoes, ticks, and rodent excrements [16].

4.2.4 Reoviridae

Colorado tick fever is the main viral disease caused by a virus of the *Reoviridae family,* transmitted by the tick *Dermacentor andersoni*. The symptoms of the infection are mild, and the course is benign in most cases. However, children under 10 years of age may develop encephalitis and hemorrhages [17].

5. Dengue

The dengue virus comprises four serotypes: DENV-1, DENV-2, DENV-3, and DENV-4. It is a virus belonging to the *Flaviviridae family*, which also includes the yellow fever virus, for example. The dengue virus is enveloped and spherical, with single-stranded RNA [3, 8]. All dengue virus serotypes are transmitted by *Aedes aegypti* or *Aedes albopictus mosquitoes* [18]. The infection can be asymptomatic or present with a wide range of clinical manifestations, including mild febrile illness to life-threatening shock syndrome. Numerous viral, host, and vector factors are believed to have an impact on the risk of infection, disease, and disease severity.

Regarding the pathophysiology of the infection, after inoculation of the virus by the vector, the dendritic cells of the skin become infected, thus migrating to the lymph nodes. *Subsequently,* lymph node migration appears viremia and the acute febrile condition. The invasion of dendritic cells by the virus causes

protein-dependent activation of TCD4 and TCD8 lymphocytes, since dendritic cells behave as antigen-presenters, as well as greater immunogenicity. It is believed that the clinical manifestations are due to the release of cytokines, mainly interferon gamma, responsible for bone marrow suppression and drop in platelet count. Viremia can also lead to invasion of the spleen, liver, bone marrow, lung, heart, gastrointestinal tract, and central nervous system [9, 10, 19].

5.1 Clinical presentation

Classically, dengue is a disease that forms part of the wide range of hemorrhagic fevers, presenting a picture similar to that caused by other arboviruses. Clinical manifestations such as fever, accompanied by at least two of the following symptoms: headache, retro-orbital pain, myalgia, arthralgia, prostration, exanthema, nausea, or vomiting, make the diagnosis probable when it occurs in an area with an epidemiological presence of the disease or with a travel history in the last 14 days to an endemic dengue area [10].

The dengue virus behaves similarly to that of yellow fever, with phases of viremia, remission, and disease status. Infection is limited by humoral immunity, which controls the invasion of new cells, and cellular immunity, which eradicates intracellular infection. The dengue virus does not have a specific tropism for a body organ, and what we witness in an epidemic is a variety of clinical conditions such as rash, headache, fever, abdominal pain, diarrhea, or involvement of the respiratory tract; frames that can occur in association or individually. Tissue damage is the result of the cytopathic effect of the virus on infected tissue.

Dengue hemorrhagic fever is a more serious condition that, although not entirely understood, is associated with a second infection by another serotype of the dengue virus, which suggests that there is an immune mechanism much more present than just the viral cytopathic effect. Other conditions associated with an evolution to dengue hemorrhagic fever are serotype 2 infection, malnutrition, black race, and adulthood. It occurs in less than 1% of dengue cases, consisting of fever, hemorrhagic phenomena, hemodynamic instability, and hepatomegaly. Clinical phenomena derive from increased capillary permeability and thrombocytopenia. Increased capillary permeability leads to hypotension and severe shock *(dengue shock syndrome),* with mortality greater than 12% under optimized therapeutic conditions. Thrombocytopenia may occur in non-severe forms of dengue, which may produce petechiae and ecchymosis; however, in dengue hemorrhagic fever, thrombocytopenia is intense, leading to hematemesis, melena, and metrorrhagia. Hepatic and central nervous system involvement occurs but not as a dominant condition in severe forms of dengue [11].

6. Dengue encephalitis

Although the dengue virus has a high prevalence, especially in countries with tropical and subtropical climates, encephalitis is a relatively little described complication [20]. The involvement of the central nervous system by any of the 4 dengue serotypes is considered uncommon in the medical literature, from encephalitis to polyneuropathies such as Guillan Barre [8].

Viral invasion of the central nervous system (CNS) can result in several clinical syndromes, including encephalitis, the core of this chapter, as well as presentations such as meningitis, myelitis, and neuritis [3]. Encephalitis is defined as an

inflammatory process of the brain parenchyma associated with clinical/laboratory evidence of neurological dysfunction.

The presence or absence of normal brain function is the important distinguishing feature between encephalitis and meningitis. Patients with meningitis may appear lethargic, yet their brain function is preserved. In encephalitis, abnormalities in brain function are a distinguishing feature, including altered mental status, motor or even sensory deficits, altered behavior, and speech or movement disturbances may be present. Other neurological manifestations of encephalitis may include hemiparesis, flaccid paralysis, and paresthesias [2].

However, the distinction between the two clinical entities is often unclear, given that parenchymal and meningeal processes can occur concomitantly. Some viral agents are more likely to cause aseptic meningitis, and others are more likely to cause encephalitis. Patients with encephalitis may have hallucinations or even be in a psychotic state. Eventually, focal or generalized epileptic seizures occur, depending on the severity of the encephalitis. Commonly, aphasia, myoclonic jerks, and focal deficits are also observed [2].

Regarding the pathophysiological mechanisms, we found some proposed theories. Firstly, a form of encephalopathy due to liver failure and release of neurotoxins and intraparenchymal hemorrhage due to extravasation and failure in the production of coagulation factors are described [19]. Secondly, supported by autopsy findings, direct neurotropic infection and local immune response occur, developing the symptoms of encephalitis due to direct neurological injury similar to other viruses of the *Flaviviridae family* [12]. In the third, an indirect, post-infectious immune response mechanism due to inflammatory factors filtered in the CSF explains the demyelinating lesion [6].

6.1 Diagnosis

The diagnosis of dengue encephalitis is challenging due to its low prevalence, leading to low suspicion and underdiagnosis. Furthermore, the clinical manifestations and images are nonspecific, and its diagnosis is based on a set of clinical, serological, and radiological findings.

Infection of the central nervous system can be confirmed by the presence in serum of IgM antibodies (dengue IgM), viral antigens (NS1), or viruses through the polymerase chain reaction (PCR) technique, where the viral RNA is identified, associated with neurological symptoms. In relation to imaging findings, there are few descriptions, with the most common findings related to changes identified in the basal ganglia, thalamus, cerebral cortex, cerebellum, and white matter, mainly in magnetic resonance imaging studies, showing restriction of diffusion by DWI and foci of hemorrhage in SWI sequence with low enhancement [19].

Other diagnoses must be ruled out to confirm the diagnosis of dengue encephalitis. In regions where dengue is endemic, a prevalence of 75% of encephalitis due to dengue has been recorded, selecting cases of meningitis and encephalitis with normal cellularity. The classic symptoms of encephalitis are a lowered level of consciousness, headache, and convulsive crises [20].

Alterations in liver function in severe dengue can also generate encephalopathy due to nitrogenous slags, with the increase in liver enzymes and changes in function markers part of the clinical picture, leading to changes in the level of consciousness, changes in behavior and cognition, and convulsions. Another rare manifestation, however, already described, of involvement of the central nervous system by the

dengue virus is acute disseminated encephalomyelitis, where the inflammatory response causes acute demyelination of a monophasic course, mainly in the white matter region, usually in the dengue recovery phase [6].

Guillain-Barré syndrome is the most common neuromuscular alteration related to dengue fever. Studies have already demonstrated high incidences, around 30%, of cases of dengue with neurological involvement.

Regarding the treatment of dengue encephalitis, there is no specific targeted therapy to date. Symptom control is carried out with the use of antiemetics and non-steroidal analgesics, and convulsive crises are aborted with classic anticonvulsants, such as benzodiazepines, barbiturates, phenytoin, and prophylaxis performed with valproic acid (always remembering liver monitoring). Case reports have shown a good response to treatment with corticosteroid pulse therapy (dexamethasone or methylprednisolone). Conditions that evolve with Guillain-Barré syndrome seem to benefit from the use of intravenous immunoglobulin, but clinical studies have not yet been carried out [12, 20].

7. Final considerations

Faced with a clinical picture with the causal possibility of encephalitis, considering epidemiological, clinical, environmental issues, among others, the arboviruses should be considered in the panel of diagnostic possibilities, and among them dengue. It is noteworthy that much of its low incidence and prevalence in endemic areas can be attributed to the diagnostic bias of the lack of consideration of such causative agents. Regarding treatment, there is no specific therapy, except, yes, general care and life support. And given the magnitude of the aforementioned pathology, as well as its various complications, it is necessary to tirelessly search for preventive measures, the most efficient and not yet available being the vaccine.

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Section 4

Diagnosis and Treatment

Chapter 8

Diagnosis of Viral Families Using a Nucleic Acid Simplification Technique

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Abstract

We have developed a novel strategy to simplify microbial nucleic acids termed 3base™. This technology uses the chemical sodium bisulphite to reduce the genome from adenine, cytosine, guanine, and thymine or uracil, in the case of RNA containing viruses, to adenine, guanine and thymine thus reducing genome complexity. The method has been applied to the detection of high-risk human papilloma virus (HPV), gastrointestinal pathogens, alphaviruses, flaviviruses, dengue and more recently coronaviruses. Currently, there are very few real-time RT-PCR based assays that can detect the presence of all members of these viral families using conventional approaches. This strategy allows the design of assays that are capable of pan-family detection. The pan-viral assays provide a sensitive and specific method to screen and thereafter speciate viral families in clinical samples. The assays have proven to perform well using clinical samples and additionally during an outbreak of dengue fever that occurred in 2016/17 on the islands of Vanuatu. The 3base™ assays can be used to detect positive clinical samples containing any viral family generally in less than 3 hours making them ideally suited to viral surveillance and perhaps the discovery of emerging viruses in families without prior sequence knowledge of the pathogen.

Keywords: human papilloma virus, gastrointestinal pathogens, flavivirus, alphavirus, dengue, coronavirus, simplification, RT-PCR

1. Introduction

Many viruses are members of large families in which the individual viruses can be diverse at the molecular level. For example, SARS-CoV-2 belongs to the family *Coronaviridae* that contains 4 distinct genera namely the Alphacoronavirus, Betacoronavirus, Gammacoronavirus and Deltacoronavirus. Many other viruses such as *Flaviviridae* and *Togaviridae* again contain many individual viruses in their designated family (see **Table 1** for examples).

Due to the genomic heterogenicity of viral families virtually all molecular diagnostic tests target individual viruses for disease diagnosis. However, even this can be challenging as viruses such as Influenza A contains many different strains based on the composition of their haemagglutinin and neuramidase genes. This chapter

Family	Genera	Notable pathogens	Species
Coronaviridae	Alphacoronavirus, Betacoronavirus, Deltacoronavirus and Gammacoronavirus	SARS-CoV-2, SARS, MERS	46
Filoviridae	Cuevavirus, Dianlovirus, Ebolavirus and Marburgvirus	Ebola, Marburg	12
Flaviviridae	Flavivirus, Hepacivirus, Pegivirus and Pestivirus	Zika, dengue, West Nile virus, Japanese encephalitis virus, yellow fever virus, Kyasanur forest disease, Alkhurma disease, Omsk hemorrhagic fever	54
Orthomyxoviridae	Alphainfluenzavirus, Betainfluenzavirus, Gammainfluenzavirus, Deltainfluenzavirus, Isavirus, Quaranjavirus and Thogotovirus	Influenza A, Influenza B	8
Papillomaviridae	53 members	HPV16, HPV18	>100
Picornaviridae	68 members	Enterovirus, Poliovirus, Hapatovirus	158
Poxviridae	Chordopoxvirinae and Entomopoxvirinae	Smallpox, Monkeypox, Cowpox	23
Rhabdoviridae	Alpharhabdovirinae, Betarhabdovirinae, Gammarhabdovirinae (plus 6 unassigned)	Lyssavirus	33
Togaviridae	Alphavirus	Chikungunya, Western Equine Encephalitis virus	32

Table 1.
Examples of the diversity contained within a number of different viral families.

describes a novel genomic simplification technique that enables the use of pan-family primers and probes to detect the presence of viral pathogens such as high-risk HPV, gastrointestinal pathogens, flavivirus, alphavirus, dengue and coronaviruses in clinical samples.

2. 3base™ a novel RNA simplification method

In order to simplify and improve the detection of viral families in clinical samples, we have developed an assay that is able to detect the presence of any high-risk HPV, gastrointestinal pathogen, flavivirus, alphavirus, dengue or coronavirus virus using a single primer and probe set for each type. These assays are based on the use of the chemical sodium bisulphite to reduce the complexity of genomes from 4 to 3 bases by deaminating cytosine to an uracil intermediate. The deamination reaction of cytosine to uracil was first described in 1970 by Hayatsu [1, 2] and has been studied in detail since. The first step of the reaction involves the sulphonation of cytosine to cytosine sulphonate followed by deamination to an uracil sulphonate intermediate and subsequently the removal of the sulphate adduct to uracil, traditionally by the use of strong alkali (**Figure 1**).

Sulphonated uracils are unable to be copied by DNA polymerases [3] due to steric hindrance as a result of the presence of the sulphate group at the C6 position. This causes distortions of DNA geometry and reduced stacking interactions [4]. Therefore, this adduct has to be removed if the resulting template is to be copied by a polymerase or reverse transcriptase enzyme. The bisulphite reaction was further refined in 1992 by Frommer and her colleagues [5] and used to differentiate cytosine from 5-methyl-cytosine in mammalian DNA as 5-methyl-cytosine is resistant to the deamination reaction. Since the publication of the genomic sequencing method, it has become the gold standard for studying the presence of methylated cytosine residues in the human genome (**Figure 2**).

However, this method resulted in up to 96% degradation of the DNA template [6] and would completely destroy RNA due to the need to desulphonate the uracil adduct with strong alkali. We have subsequently refined the method so that the degradation of DNA and RNA has been eliminated allowing "simplification" of both microbial DNA and RNA.

The 3base™ protocol deaminates all cytosine residues in nucleic acid to uracil, which are subsequently copied as thymine by a polymerase (**Figure 3**) or reverse transcriptase enzyme [5]. After simplification individual species become more similar in base composition resulting in reduced complexity of primer and probe sets for pan-family identification. The resulting primer and probe sets have fewer mismatches to the original sequences thus allowing binding of these to regions of nucleic acid that were previously heterogeneous in nature. The use of the simplification method does not result in a loss of specificity as it is still possible to design individual primer sets that can detect the viral species responsible for disease.

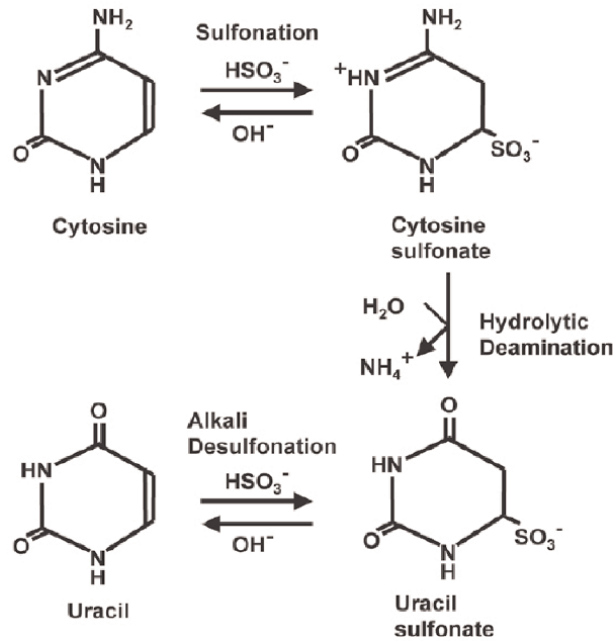

Figure 1.
Shows the reaction of cytosine with sodium bisulphite.

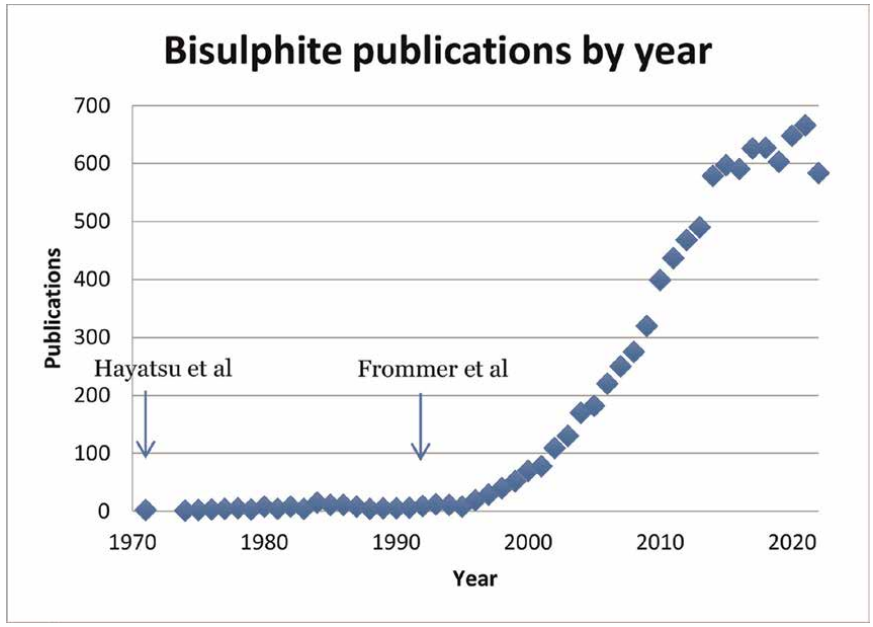

Figure 2.
Schematic representation of published papers using the bisulphite method with arrows representing the publication of the description of the cytosine deamination method and the bisulphite sequencing protocol.

3 base™ bisulphite mechanism

Before	C G T A G C C T C A C T T C C A G G A C T G G C	
After	T G T A G T T T T A T T T T T A G G A T T G G T	
	Before bisulphite	After bisulphite
Seq#1	GATGGCGATATGGTTGACAC	GATGGTGATATGGTTGATAT
Seq#2	GATGGTGACATGGTAGATAC	GATGGTGATATGGTAGATAT
Seq#3	GATGGTGATATGGTGGACAC	GATGGTGATATGGTGGATAT
Seq#4	GATGGTGATATGGTAGATAT	GATGGTGATATGGTAGATAT
Seq#5	GATGGTGATATGGTGGACAC	GATGGTGATATGGTGGATAT
Seq#6	GATGGCGACATGGTTGATAT	GATGGTGATATGGTTGATAT
Seq#7	GATGGTGATATGGTGGACAC	GATGGTGATATGGTGGATAT
Seq#8	GATGGTGACATGGTAGATAC	GATGGTGATATGGTAGATAT
Seq#9	GATGGTGATATGGTAGATAC	GATGGTGATATGGTAGATAT
Seq#10	GATGGTGATATGGTGGATAC	GATGGTGATATGGTGGATAT
Consensus	GATGGYGAYATGGTDGAYAY	GATGGTGATATGGTDGATAT
	75% homology over 20 bases	95% homology over 20 bases
	48 possible combinations	3 possible combinations

Figure 3.
Shows the simplification process where cytosine residues are converted to uracil.

3. Viral surveillance

New viruses will continue to appear due to evolutionary pressure, climate change and the demise of natural habitats as a result of human intervention. The Zika virus epidemic that began in 2016 demonstrates that a flavivirus originally thought to be relatively benign can emerge as a significant public health threat within a relatively short space of time [7]. SARS-CoV-2 emerged in 2019 and subsequently gave rise to a global pandemic which to date has resulted in over 620,000,000 confirmed cases and over 6,500,000 deaths [8]. SARS-CoV-2 more than likely emerged from an animal reservoir, and it is highly likely that other coronavirus threats will emerge in the future likely by the same route. While it is impossible to predict the rise of a particular virus in the human population it is almost certain that in the future new viral threats will emerge which will more than likely result in widespread morbidity and mortality.

As a result of the recent SARS-CoV-2 pandemic it is likely that governments will in the future invest in a more extensive network of testing equipment, stockpile reagents and enable easier regulatory protocols. While this could reduce the time required for testing, a critical phase exists of when a new pathogen becomes infectious to the general population, and when reliable diagnostic tests are generally available. A strategy that may allow for less-restricted screening for novel pathogens during this period is the use of pan-family assays: molecular diagnostic tests which target a family of viruses rather than a single species [9].

The use of species-specific PCR is unlikely to pick up new strains of a virus and this was demonstrated in the case of SARS-CoV-2, which was only detected on Next Generation Sequencing (NGS) and not by conventional PCR using species-specific primers and probes [10]. Interestingly, we had already developed a pan-coronavirus 3base™ assay that on publication of the complete genome of SARS-CoV-2 [11] would have picked up this variant without prior knowledge of the viral genomic sequence. The pan-family PCR approach is thus perhaps a simpler and more cost-effective alternative to NGS for viral surveillance?

It has been postulated that one of the more obscure viruses in the *Flaviviridae* family such as Spondweni virus (SPOV), Usutu virus (USUV), Ilheus virus (ILHV), Rocio virus (ROCV), Wesselsbron virus (WSLV) or tick-borne flaviviruses may be the next pathogen to emerge into the human population [7]. The use of the pan-flavivirus 3base™ assay would be the ideal tool to screen for emerging flaviviruses entering the population without the expense and labour costs of screening each and every sample for all of the individual flavivirus species that are currently known.

4. Pan viral diagnosis

There are several methods available for molecular pan-viral diagnosis (see **Table 2**). Perhaps the first was the use of arrays fabricated with large numbers of oligonucleotides probes specific for individual pathogens. Hybridisation of a clinical sample to such arrays was then be used to detect the presence of viral genomes in infected individuals [39]. However, using this approach prior sequence knowledge of the pathogens are required to design the specific oligonucleotides to be arrayed, and as stated previously emerging pathogens are highly likely to contain divergent nucleic acid sequences. Another approach for the detection of novel pathogens is the use of Next Generation Sequencing (NGS). Unlike the array approach no prior knowledge of an emerging viral sequence is required as all nucleic acids in the sample can be

Pan-virus target	Method	Reference
Adenovirus	RT-PCR	Kosulin et al. [12]
Bluetongue virus	RT-PCR	Mulholland et al. [13]
Coronavirus	RT-PCR	Erlichster [9], Holbrook [14]
Dengue	RT-PCR	Hu et al. [15], Waggoner et al. [16], Simmons et al. [17], Waggoner et al. [18]
Filovirus	RT-PCR	Jääskeläinen et al. [19]
Flavivirus	RT-PCR	Khongwichit et al. [20]
Foot and mouth disease	RT-PCR	Bachanek-Bankowska et al. [21]
Hepatitis B	Numerous (review article)	Wose Kinge [22]
Hepatitis C	RT-PCR	Walker et al. [23]
lyssavirus	RT-PCR	Marsten et al. [24], Condori et al. [25], Fischer et al. [26]
Orthopox	PCR	Grant et al. [27]
Phlebovirus	RT-PCR	Klimentov et al. [28]
Pegivirus	Microarray	Fridholm et al. [29]
Poxvirus	RT-PCR	Li et al. [30]
HPV	PCR	Chang et al. [31], Chouhy et al. [32]
Paramyxovirus	RT-PCR	Schatzberg et al. [33]
Simbu virus	RT-PCR	Fischer et al. [34]
Viral meningitis	NGS	Guan et al. [35]
Viral pathogens	Microarray	Chen et al. [36]
Viral pathogens	Microarray (genus specific oligonucleotides)	Kang et al. [37]
Viral pathogens	Microarray	Tang et al. [38]

Table 2.
Examples of the pan-family approach applied to molecular diagnostics.

sequenced then assembled by alignment with established genomes to produce a best match. Recently the costs associated with NGS have reduced dramatically from when the technology was in its infancy thus it is now possible to apply this technique to viral discovery [40]. However, the use of viral arrays and NGS is still more costly, labour intensive and less sensitive compared to the more routine technique of RT-PCR which can generate clinically meaningful data in around 1 h.

Many viruses that infect humans cause non-specific symptoms such as headache, fever, arthralgia, myalgia, and lethargy making initial diagnosis based on clinical symptoms challenging. This is especially true of respiratory viruses thus pan-family diagnosis can reduce the number of primer and probe sets that are require for molecular syndromic testing. **Table 3** shows that if the pan-family approach was used for respiratory viruses screening the number of individual reactions that would be required to identify the infectious agent is reduced from 20 to 7 reducing costs and the labour involved. After identifying the family responsible for infection individual typing primers could then be used to detect the exact species if required. Likewise,

Species-specific approach	Pan-family approach
Influenza A virus (Flu A)	Pan-orthomyxoviridae
Influenza A-H1 (Flu A-H1)	
Influenza A-H1pdm09 (Flu A-H1pdm09)	
Influenza A-H3 (Flu A-H3)	
Influenza B virus (Flu B)	
Respiratory syncytial virus A (RSV A)	Pan-orthopneumovirus
Respiratory syncytial virus B (RSV B)	
Parainfluenza virus 1 (PIV 1)	Pan-parainfluenza
Parainfluenza virus 2 (PIV 2)	
Parainfluenza virus 3 (PIV 3)	
Parainfluenza virus 4 (PIV 4)	
Coronavirus 229E (229E)	Pan-coronavirus
Coronavirus NL63 (NL63)	
Coronavirus OC43 (OC43)	
Coronavirus HKU-1 (HKU-1)	
SARS-CoV-2	
Human rhinovirus (HRV)	Pan-picornavirus
Enterovirus (HEV)	
Adenovirus (AdV)	Pan-Adenovirus
Metapneumovirus (MPV)	Metapneumovirus

Table 3.
Shows that using the pan-family screening approach the number of individual reactions required for a comprehensive respiratory screen is reduced from 20 to just 7.

infection with arboviruses manifest in similar symptoms thus the use of the pan-family screen can provide a rapid diagnosis of the family involved without the need to perform multiple individual PCR reaction to determine the cause of infection. After determination of the species responsible for infection again samples can then be typed using species-specific PCR if required.

5. Human papilloma virus (HPV)

The family *Papillomaviridae* contains a group of double stranded DNA viruses containing a circular genome of approximately 8000 base pairs [41, 42] that were first described to be associated with skin warts in 1907 [43]. The family papillomavirus contains over 100 individual members many of which cause no symptoms with the vast majority (90%) resolving after 2 years [44]. HPV can infect many different sites in the body including the skin, throat, tonsils, mouth, cervix, vulva, vagina, penis, and anus.

It was first postulated in 1976 that HPV could be associated with the development of cervical cancer [45]. Genital HPV infection can be caused by at least 50 individual

	Low risk	Probable high risk	High risk	Highest risk
HPV type	6, 11, 42 and 44	26, 53, 66, 68, 73 and 82.	33, 35, 39, 51, 52, 56, 58 and 59	16, 18, 31 and 45

Table 4.
HPV viruses classified according to the risk of cervical cancer development.

viruses that can be split into four classes as shown in **Table 4** [46]. The high-risk types of HPV have been shown to be associated with the development of cervical cancers [47–49].

Traditional methods for the diagnosis of cervical cancer have relied heavily on cytology in which cells of the cervix are observed under the microscope for the presence of cancerous or precancerous lesions. This test, known as the Papanicolaou (Pap) test was invented in the 1920s by Georgios Papanikolaou and Aurel Babeș and subsequently named after Papanikolaou. A simpler version of the test was discovered by Anna Marion Hilliard in 1957. The use of the Pap test when used in combination with molecular methods has been shown to increase the sensitivity in which pre-cancerous lesions can be detected in the cervix [50].

5.1 Molecular detection of HPV

There are a number of molecular methods that can be used to detect the presence of HPV in clinical samples [51–55]. One common primer pair, the MY set, was first described in 1989 [56] detects a common region of the viral L1 gene that is found in all HPV types. Improvements on these primers generated the GP set that are able to detect more strains of the virus [57]. However, these primer sets are unable to differentiate the presence of high-risk HPV from low risk therefore amplicons must be sequenced or hybridised to oligonucleotide arrays to determine the strain of the virus responsible for infection.

One of the earliest FDA approved molecular tests for HPV was the hcII HPV test (Digene Corporation, USA). This test used oligonucleotide probes that were specific for each of the high and low risk viruses. The method was based on capture of specific HPV sequences present in the clinical sample coupled with a chemiluminescent read-out. However, it has been demonstrated that this assay could generate both false positive and negative results [58].

5.1.1 3base™ detection of high-risk HPV types

To produce an assay capable of detecting specifically the high-risk viruses we aligned the sequences of the complete genomes of the high-risk HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 along with the low-risk types 6, 11, 43 and 44 to serve as reference for non-target HPV strains. Using this approach, we were able to design a nested PCR assay that was specific for high-risk HPV types only. The primers were tested extensively on a large number of previously typed ThinPrep® liquid-based cytology samples to ensure that the assay was specific for the high-risk-types.

5.1.2 3base™ high-risk HPV clinical trial

A total of 834 ThinPrep® samples were tested using the 3base™ simplification method and compared to the reference hcII method. Discordant samples were the

amplified using a reference method (MY09/MY11 and the GP5+/GP6+ primer sets) and the amplicons subsequently sequenced to identify the strain of virus present. As can be seen from **Table 5** both methods demonstrated a similar sensitivity which was not statistically significant (p = 0.398). However, the specificity of the 3base™ was significantly higher than the hcII method (p = 0.001) and as would be expected the PPV for the 3base™ test was also significantly higher [59].

	Reference method (PCR)					
	Positive	**Negative**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**
3base™ method						
Positive	197	49	63.1	90.6	80.1	80.4
Negative	115	473				
Digene hcII method						
Positive	202	80	64.7	84.6	71.6	80.1
Negative	110	442				

Table 5.
The results generated in an independent clinical trial comparing the 3base™ to a commercially available assay for the detection of HPV in cervical samples.

6. Gastrointestinal disease

Gastrointestinal (GI) diseases occur globally and are a major cause of morbidity and mortality. In developed countries the mortality due to GI disease is relatively low compared to that of developing countries [60]. It has been estimated that in developing countries up to 2 million children under the age of five die from GI infections annually [61].

There are many viral, bacterial, and protozoan agents that are responsible for gastroenteritis in humans. Perhaps the most notable of these are the viral agents that include Norovirus, Rotavirus, Sapovirus, Astrovirus and the Adenoviral group. One of the most common causes of gastroenteritis is Norovirus which is responsible for outbreaks of disease especially in children with an estimated 685 million cases and around 200,000 deaths occurring annually worldwide [62].

The viruses that cause gastroenteritis are a diverse group of pathogens with many genotypes and genogroups responsible for disease (see **Table 6** below).

There are also a wide range of bacterial species that are responsible for gastrointestinal illness with the most common agents that of *Campylobacter* spp. and *Salmonella*. The CDC estimated that in the USA that 43% of bacterial gastrointestinal cases are caused by *Salmonella* spp. followed by *Campylobacter* spp. representing a further 33% [66]. Other notable causes of GI disease are *Shigella* spp., *Yersinia enterocolitica*, *Clostridium difficile* and pathogenic strains of *Escherichia coli*. Different bacterial agents also show distinct geographical distributions with species such as *Vibrio cholera* and *Shigella* spp. more common in developing countries [67].

Protozoan species also contribute to the burden of GI diseases with notable agents such as *Giardia* spp., *Cryptosporidium* spp. and *Enteramoeba histolytica* the most

Virus	Genome	Genotypes	Genogroups	Ref
Norovirus	Positive sense RNA	10	49	Chhabra et al. [63]
Rotavirus	Double stranded RNA	7	36G and 51P	Wahyuni et al. [64]
Sapovirus	Positive sense RNA	19		Tang et al. [65]
Astrovirus	Positive sense RNA	8 (+2 novel)		Tang et al. [65]
Adenovirus	Double stranded DNA	7		Tang et al. [65]

Table 6.
The diversity of viral agents responsible for GI disease.

common causes. Other agents such as *Dientamoeba fragilis* and *Blastocystis hominis* have also been implicated in the aetiology of gastroenteritis [68–70].

The symptoms of gastrointestinal disease can include diarrhoea, vomiting, abdominal pain, fever, general lack of energy, and dehydration. These symptoms are shared between the many organisms that cause symptoms thus traditionally disease was diagnosed by a combination of culture, microscopy and EIA for bacteria, protozoan and viral disease respectively. These techniques are laborious and in some cases such as conventional culture can take up to 4–5 days to yield positive results [71].

To simplify the detection of gastrointestinal pathogens and streamline the process we sought to use 3base™ technology to not only detect the complex viral causes of gastroenteritis but also the individual bacterial and protozoan agents responsible for gastroenteritis [72].

6.1 Viral pathogens

Sequences for all genotypes of Norovirus, Rotavirus, Astrovirus, Sapovirus and Adenovirus were downloaded from the NCBI nucleotide database and aligned using the free web-based alignment tool Dialign (https://dialign.gobics.de). Regions were then chosen to produce primer and probe sets to amplify each viral group. After initial optimisations the best sets were used to screen a bank of archived clinical samples with the results are shown in **Table 7**.

As can be seen from **Table 7** the assay was able to detect the presence of all viral targets. In addition, the assay was validated independently yielding similar results.

6.2 Bacterial and protozoan pathogens

Although bacterial and protozoan causes of gastroenteritis are not as complex as the viral targets it was important that the assay was able to detect these pathogens as gastroenteritis is a syndromic disease. **Tables 8** and **9** demonstrate the ability of the 3base™ method to detect organisms at the species level.

As can be seen from the data the 3base™ assay does not suffer from a loss of specificity when primer and probe sets are designed to detect organisms at the species level. The syndromic multiplex PCR assay is thus a useful tool for the detection of viral, bacterial, and protozoan causes of gastroenteritis without the need for time consuming and labourious conventional methods. In addition, testing can be centralised with a turnaround time of less than 4 h.

Viral specimens (n = 109)			3rd party evaluation	
Species	**EIA**	**3base™ assay**	**Conventional**	**3base™ assay**
Norovirus	81	81	15	16
Rotavirus	21	21	15	15
Astrovirus	5	5	1	3
Adenovirus	2	2	2	2
Sapovirus	0	0	0	0

Table 7.
Results generated using the 3base™ on stool samples with viral gastroenteritis.

Bacterial specimens (n = 80)			3rd party evaluation	
Species	**culture**	**3base™ assay**	**Conventional**	**3base™ assay**
Campylobacter spp.	40	41	13	13
Salmonella spp.	32	31	5	5
C. difficile	4	4	17	18
Shigella spp.	1	1	2	2
Y. entercolitica	0	0	2	2
Listeria monocytogenes	0	0	0	0
Negative	3	3		

Table 8.
Detection of bacterial causes of gastroenteritis.

Parasite specimens (n = 81)			3rd party evaluation	
Species	**Microscopy**	**3base™ assay**	**Conventional**	**3base™ assay**
G. intestinalis	33	37	2	4
Cryptosporidium spp.	15	15	0	0
D. fragilis	12	13	4	5
Entamoeba complex	N/A	7	0	0
E. histolytica	0	0	0	0
B. hominis	15	20	2	3
Negative	11	6		

Table 9.
Detection of protozoan causes of gastroenteritis.

7. Coronaviridae

The Coronavirus family members are sub classified as alpha, beta, gamma and deltacoronaviruses [73, 74]. Alphaconoronaviruses contain least 10 known species including human coronavirus (hCoV) 229E that causes the common cold, many bat, feline, canine coronaviruses, and the porcine transmissible gastroenteritis coronavirus. The Betaconoronaviruses contain members such as SARS-CoV-1,

MERS-CoV, human coronavirus 0C43 and now SARS-CoV-2. The Gammacoronaviruses genera contain avian, duck coronavirus and the infectious bronchitis virus. Finally, the deltacoronaviruses members include HKU11, HKU12, HK13 that cause the common cold. **Figure 4** illustrates a phylogenetic tree showing the relatedness of various coronavirus strains. Most of the members of the coronavirus family exhibit a zoonotic lifecycle, that in rare occasions result in a spill over event to the human population.

A number of notable human coronaviruses have emerged in the last two decades which can result in severe respiratory disease. The severe acute respiratory syndrome (SARS) originated as a mystery illness in Guangdong, China in 2002 and resulted in an epidemic that killed 10% of the 8000 people it infected [75]. The etiological agent was subsequently identified as the severe acute respiratory syndrome coronavirus (SARS, now renamed SARS-CoV-1). This was the fifth hCoV to be identified and is thought to have originated as an animal virus from an unknown animal reservoir. The disease was characterised by flu-like symptoms, high fevers exceeding 38°C, myalgia, dry non-productive cough, difficult breathing, and an infiltrate seen on chest radiography.

Ten years later in 2012, a sixth hCoV was isolated from a patient presenting with severe respiratory illness in Jeddah, South Arabia [76]. The etiological agent was later designated Middle East respiratory syndrome coronavirus (MERS-CoV). MERS-CoV has been detected in more than 27 countries across the Middle East, Europe, North Africa, and Asia. There has been a total of 2040 MERS-CoV laboratory confirmed cases, with 712 deaths (34%) making this the most lethal coronavirus to date.

Another novel coronavirus (SARS-CoV-2) emerged into the human population in December 2019 in Wuhan, China, and has subsequently become the deadliest coronavirus to emerge in the human population in the past two decades [77], bringing the number of hCoV to seven. The disease (Covid-19) is believed to have been contracted from an animal virus that crossed over into the human population, more than likely from bats. The virus has spread globally and infected over 620,000,000 people resulting in over 6,500,000 deaths [8] which although far more than the MERS-CoV epidemic represents only a 1% case fatality rate compared to 34% for MERS-CoV. Such a large-scale spread is a result of efficient human-human transmission as the virus evolves to improve its ability to infect its human host.

The severity of Covid-19 and the rapid spread of the virus is a wakeup call to rethink diagnostic approaches, especially for the coronavirus family that has many members maintained by a variety of animal reservoirs such as bats, birds, pangolins, and snakes [78–80]. Covid-19 is an example of what can happen if a spill over event involves a virus well attuned to human-human transmission. The severity of coronavirus disease and the potential for new emerging viruses calls for rapid diagnostic tests which can quickly and accurately detect these viruses in clinical samples and animal hosts. The pan-family molecular approach could be an ideal method to screen for coronaviruses in general and detect novel strains as they emerge.

7.1 Design of the pan-coronavirus assay

Whole genomic sequences of SARS-CoV-1, MERS, HKU-1, NL63, 229E and OC43 were downloaded from the data base and aligned using the Geneious Prime™ software to generate optimal regions for the design of 3base™ primers and probes. These were them tested using synthetic constructs to determine assay sensitivity and specificity (see **Table 10**).

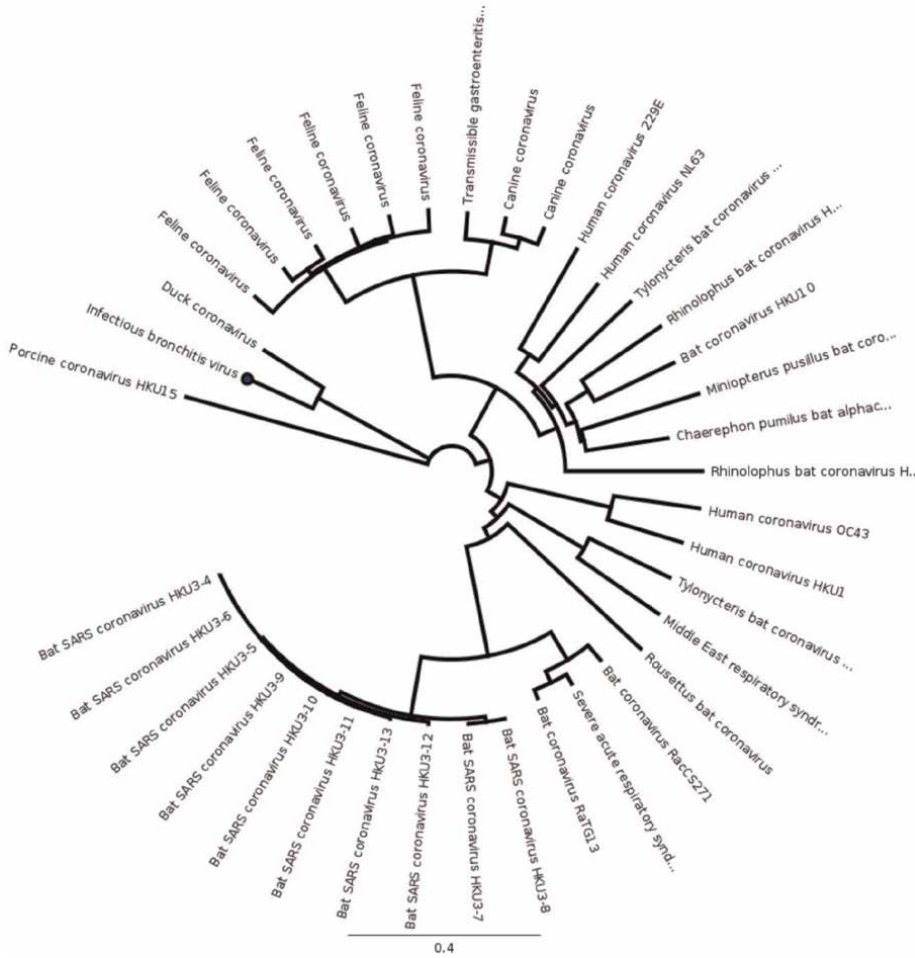

Figure 4.
Shows a phylogenetic tree generated using whole genomes of various coronaviruses using the Geneious prime™ tree building software.

After initial assessment and assay validation including cross-reactivity studies the pan-coronavirus component was then multiplexed with the SARS-CoV-2 E and N genes for clinical studies using cultured SARS-CoV-2 virus. **Table 11** shows that the triplex assay could detect low levels of SARS-CoV-2 virus.

The clinical performance of the assay was established using 1, 662 patient samples sourced from a local hospital in 2020 when the virus was still relatively rare in Australia. Twenty-five samples were found to be positive for SARS-CoV-2 by both the pan-coronavirus and gene specific assays. In addition, a further 45 samples were positive using the pan-coronavirus assay and negative with the SARS-CoV-2 specific primer and probe sets. These samples were then tested with a confirmatory assay that detected the presence of seasonal coronaviruses. This assay detected 37 samples as either NL63, 229E, OC43 or HKU-1. Of the eight samples that were negative by the confirmatory assay, five were available for sequencing using the pan-coronavirus amplicons. On sequencing the results showed that these samples contained a novel HKU-1 variant not targeted in the confirmatory test.

	Pan-Coronavirus (replicates positive)						
Copies/PCR	**MERS**	**SARS-CoV-2**	**SARS-CoV-1**	**NL63**	**229E**	**HKU1**	**OC43**
1E4	5/5	5/5	5/5	5/5	5/5	5/5	5/5
1E3	5/5	5/5	5/5	5/5	5/5	5/5	5/5
1E2	5/5	5/5	5/5	5/5	5/5	5/5	5/5
50	5/5	5/5	5/5	5/5	5/5	5/5	5/5
25	5/5	5/5	4/5	5/5	5/5	5/5	5/5
12.5	5/5	5/5	3/5	5/5	5/5	5/5	4/5
6.25	4/5	3/5	2/5	5/5	4/5	4/5	4/5
3.125	0/5	2/5	3/5	1/5	2/5	4/5	1/5

Table 10.
Sensitivity of the pan-coronavirus assay tested on synthetic construct.

SARS-CoV-2	**Pan-Coronavirus**	**E-gene**	**N-gene**
Dilution	Positive	Positive	Positive
1\10	2/2	2/2	2/2
1\100	2/2	2/2	2/2
1\1000	2/2	2/2	2/2
1\10,000	2/2	2/2	2/2
1\20,000	2/2	2/2	2/2
1\40,000	2/2	2/2	2/2
1\80,000	2/2	2/2	2/2
1\160,000	1/2	2/2	2/2

Table 11.
Shows the results using the pan-coronavirus triplex assay on cultured viral samples.

8. Current pan-flavivirus/alphavirus assays

A PubMed.*gov* search was performed using the keywords pan-flavivirus real time PCR (RT-PCR), pan-alphavirus RT-PCR and pan-dengue RT-PCR to determine the number of assays that employed a pan-family approach. Although this is not a definitive search the results give an idea of what is possible at present using conventional real-time PCR. From 1996 to 2022 a total of 1, 182 paper were found that used real-time PCR to detect the presence of flaviviruses in general. Of these only 2 papers used the pan-flavivirus detection approach. Similarly, from 2004 to 2022 a total of 294 papers mentioned RT-PCR for the detection of alphavirus with only 1 using a pan-species approach with this assay using multiple primers due to target sequence degeneracy. With dengue virus from 2001 to 2022 a total of 782 papers were published that mentioned dengue virus and real-time PCR with 32 using pan-dengue RT-PCR primers and probes.

As the dengue virus family contains only 4 members it makes sense that this was the target to which most pan-family assays were designed. The flavivirus and

alphavirus virus families are much more complex and contain 54 and 32 members respectively and are much underrepresented with pan-family tests compared to dengue. The reduction in the number of assays able to detect pan-flavivirus and pan-alphavirus is presumably due to sequence divergency of the individual members making the selection of suitable primers and probes for pan-family identification using conventional RNA more challenging.

This is where the use of the chemical simplification step can make the selection of regions to design primers and probes easier (see **Table 12**). As can be seen before the genomic simplification process the consensus sequence for a pan-alphavirus primer would contain a total of 576 individual primers to produce sequences that were a perfect match for all targets. However, after the simplification process the primer pool would be reduced to just 27 representing a major reduction in genomic complexity.

8.1 Flavivirus/alphavirus and dengue

The *Flaviviridae* family of viruses contain many individual members that result in a heavy toll in terms of morbidity and mortality globally on an annual basis. Notable members include dengue which has been estimated to cause over 400 million

	Sequence	
Alphavirus species	**Before conversion**	**After conversion**
Barmah Forest Virus	CCUUACUUCUGUGGAGGAUUU	TTTTATTTTTGTGGAGGATTT
Ndumu virus	CCGUAUUUCUGCGGCGGGUUC	TTGTATTTTTGTGGTGGGTTT
Chikungunya virus	CCUUACUUUUGUGGAGGGUUU	TTTTATTTTTGTGGAGGGTTT
O'nyong-nyong virus	CCAUACUUCUGUGGGGGAUUU	TTATATTTTTGTGGGGGATTT
Middelburg virus	CCCUACUUCUGCGGAGGGUUU	TTTTATTTTTGTGGAGGGTTT
Mayaro virus	CCCUACUUUUGUGGAGGUUUC	TTTTATTTTTGTGGAGGTTTT
Ross River virus	CCAUACUUCUGCGGCGGGUUU	TTATATTTTTGTGGTGGGTTT
Semliki forest virus	CCAUAUUUUUGUGGGGGAUUC	TTATATTTTTGTGGGGGATTT
Una virus	CCUUACUUCUGCGGAGGAUUC	TTTTATTTTTGTGGAGGATTT
Aura virus	CCUUACUUUUGCGGCGGAUUU	TTTTATTTTTGTGGTGGATTT
Rio Negro virus	CCAUACUUUUGUGGAGGGUUU	TTATATTTTTGTGGAGGGTTT
Mucambo virus	CCGUACUUUUGCGGCGGGUUU	TTGTATTTTTGTGGTGGGTTT
Everglages virus	CCCUAUUUUUGUGGAGGGUUU	TTTTATTTTTGTGGAGGGTTT
Venezuelan equine encephalitis virus	CCCUAUUUUUGUGGAGGGUUU	TTTTATTTTTGTGGAGGGTTT
Eastern equine encephalitis virus	CCGUACUUUUGCGGAGGGUUC	TTGTATTTTTGTGGAGGGTTT
Western equine encephalitis virus	CCCUACUUCUGUGGGGGAUUU	TTTTATTTTTGTGGGGGATTT
Consensus sequence	CCNUAYUUYUGYGGDGGDUUY	TTDTATTTTTGTGGDGGDTTT
Number of variants	**576**	**27**

The red colouring indicates bases that are degenerate in the sequences before and after the simplification process.

Table 12.
Genomic simplification of alphavirus sequences reduces the number of primer variations from 576 to just 27.

infections yearly with 100 million cases in 2010 [81]. Other species include Zika which caused epidemics between 2014 and 2017, yellow fever virus which is endemic in Africa and South America, Japanese encephalitis virus and West Nile virus which has been associated with sporadic outbreaks in the USA. Flaviviruses infections range from asymptomatic to life threatening conditions such as hemorrhagic fevers. Flaviviruses are characterised by a positive sense single stranded RNA genome that ranges in size from 10 to 11 Kb. The genome consists of 8 non-structural and 3 structural proteins [82].

Alphaviruses are members of the *Togaviridae* group of viruses with genomes of around 11–12 Kb that like flaviviruses contain a single stranded positive sense genome [83]. Alphaviruses infect a wide range of birds, fish and mammals including humans. Probably the best-known alphaviruses are chikungunya, Barmah Forest virus and O'nyong'nyong virus. Both flavi- and alphaviruses are arboviruses and are most commonly transmitted to the human population via a bite from an infected mosquito or tick.

The global distribution of flaviviruses and alphaviruses can be overlapping or unique with some viruses specific for certain geographical locations (**Figure 5**). Epidemics of flavivirus and alphavirus occur on an annual basis with different degrees of severity thus rapid and specific molecular diagnostic approaches are required to aid in patient management.

8.2 Design of 3base™ primers and probes

To determine if the pan-flavivirus, pan-alphavirus and pan-dengue simplification method could be used in screening and outbreak management we designed 3base™ assays for each family of pathogens.

8.2.1 3base™ pan-flavivirus/pan-dengue assays

The complete genomes of the following flaviviruses were analysed using Geneious software to determine the optimal regions for 3base™ primers and probes; Karshi virus (AY863002), Powassan virus (EU670438), Kyasanur forest disease virus

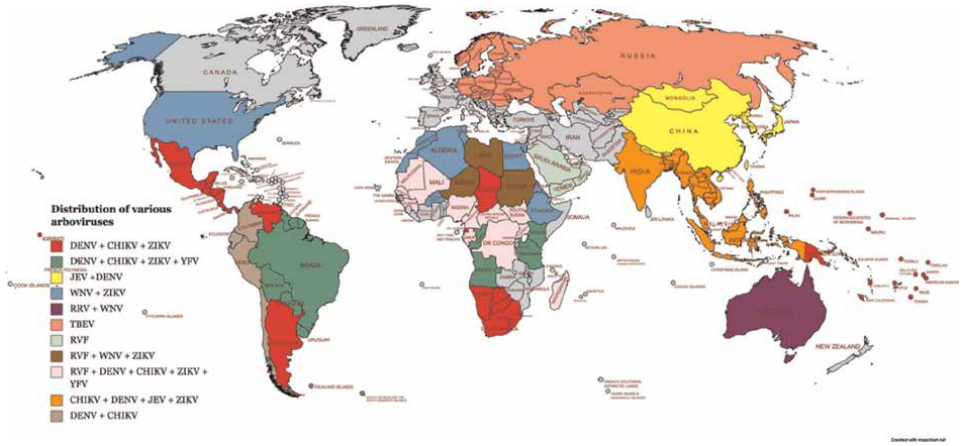

Figure 5.
Shows the global distribution of a number of important arboviruses (this map was prepared using information in Socha et al. [84] using the free web based MapChart software).

(AY323490), Langat virus (NC_003690), Omsk hemorrhagic fever virus (AB507800), Tick-borne encephalitis virus (KU761572), Yellow fever virus (MF423374), Sepik virus (DQ859063), Wesselsbron virus (JN226796), Dengue 4 (EU854296), Dengue 2 (AF038402), Dengue 3 (AB189125), Dengue 1 (AB189120), Zika (KU820899), Saint Louis encephalitis virus (MN233312), West Nile virus (KT57320), Kunjin virus (KX394405), Japanese encephalitis virus (AF080251), Usutu virus (AY453411) and Murray Valley encephalitis virus (AF161266).

8.2.2 3base™ pan-alphavirus assay

The complete genomes of the following alphaviruses were analysed using Geneious software to determine the optimal regions for 3base™ primers and probes; Barmah Forest virus (NC_001786), Ndumu virus (NC-01659), Chikungunya virus (NC_004162), O'nyong-nyong virus (NC_001512), Middelburg virus (NC_024887), Mayaro virus (NC_003417), Ross River virus (NC_001544), Semliki forest virus (NC_003215), Una virus (NC_043403), Aura virus (NC_003900), Rio Negro virus (NC_038674), Mucambo virus (NC_038672), Everglades virus (NC_038671), Venezuelan equine encephalitis virus (NC_001449), Eastern equine encephalitis virus (NC_003899) and Western equine encephalitis virus (NC_003908).

8.3 Assay performance

Numerous primer/probe sets were designed for the pan-flavivirus, pan-dengue and pan alphavirus assays and sets then wet tested to determine optimal sensitivity and specificity. After initial screening the best performing sets were tested using individual synthetic oligonucleotoides specific for each virus. The pan-flavivirus assay was able to detect the presence of DENV-1, DENV-2, DENV-3, DENV-4, TBEV, WNV, YZV and Zika virus with a lower limit of detection (LLOD) of 12.5 copies/PCR for all species tested.

Likewise, the pan-alphavirus assay was able to detect the presence BFV, CHIKV, EEEV, MVE, NV, RRV, VEEV and WEEV with a sensitivity of 10 copies/PCR for VEEV, RRV, NV, BFV and MV, 25 copies/PCR for CHIKV and EEEV and 50 copies/PCR for WEEV.

To assess potential cross reactivity with other viruses after the 3base™ simplification process a large number of RNA and DNA samples were obtained from a number of human viruses. No cross reactivity was observed with any component of the assays using a wide range of both DNA and RNA containing human pathogens.

Molecular quality assurance panels obtained from QCMD for dengue, Zika virus and chikungunya from 2016 to 2018 demonstrated that the pan-flavivirus/pan-alphavirus/pan-dengue assays were in 100% concordance with the expected results. These results indicate that the simplification assays are performing well, if not better than other molecular assays used worldwide.

8.4 Vanuatu 2016/2017 dengue outbreak

T0 date traditional methods such as Enzyme Immuno Assays (EIAs) have been the method used for the detection of both flavi- and alphaviruses. It has been shows that dengue EIAs show and high degree of cross reactivity with Zika virus and likewise

Zika EIAs cross react with dengue [85, 86]. Unlike molecular approaches conventional EIAs are unable to differentiate the individual dengue serotypes and in addition are generally less sensitive than molecular assays. However, unlike the RNA simplification approach there are very few RT-PCR assays can target all members of complex groups such as flavivirus or alphavirus using a single primer and probe set.

There have been numerous outbreaks of arboviruses in the South pacific regions. From 2012 to 2014 it was estimated that at least 28 outbreaks of disease have occurred which were attributed mainly to dengue virus but notable outbreaks as a result of chikungunya and Zika virus were also recorded [87]. These outbreaks cause severe stress on both the public health system and on the islands economy which for the most part are tourist driven.

During 2016/2017 an outbreak of dengue fever occurred on the islands of Vanuatu [88]. Vanuatu consists of a group of over 80 islands that are located in the South Pacific region the largest of which is Efate home to over 86, 000 residents. The population on the rest of the islands range from as many as 46,000 to as low as a few hundred. From the 12th to 24th March 2017, we tested both archived and fresh samples obtained from Port Villa central hospital, Efate, to determine if the 3base™ pan-flavivirus, pan alphavirus and pan-dengue assays were useful in an outbreak situation. We included a dengue 2 specific primer and probe set since this was the genotype responsible for the outbreak. Samples were extracted using a small footprint automated extraction platform along with a small portable PCR machine weighing less than 2 kg.

Over the study period we tested 187 serum sample for the presence of dengue (see **Table 13**). One hundred and sixteen samples tested positive for the presence of pan-flavivirus, pan-dengue and the specific dengue 2 assay representing a positivity rate of 62%. Seven samples were inconclusive as only signals were obtained with the pan-dengue component of the assay which could be explained by a very low viral load in these particular samples.

When we plotted the dengue positivity from December to March (see **Figure 6**) we found that the number of positive dengue cases peaked in the month of January followed by a marked decline in positivity in February. Routine testing of patients with dengue like symptoms using the pan-family assays commenced in the middle of March and we found that the number of cases began to increase again at this time. As molecular methods are more sensitive than the conventional EIA assays the rise could be attributed to increased sensitivity of the pan-family assays [80].

	Number	**% Positive**
pan-flavivirus	116	62
pan-alphavirus	0	0
pan-dengue	123	66
DENV-2	116	62
dengue not typed	7*	3.2
Negative	64	34

**Weak samples with a Ct value >40.*

Table 13.
Results of clinical sample obtained during the Vanuatu outbreak.

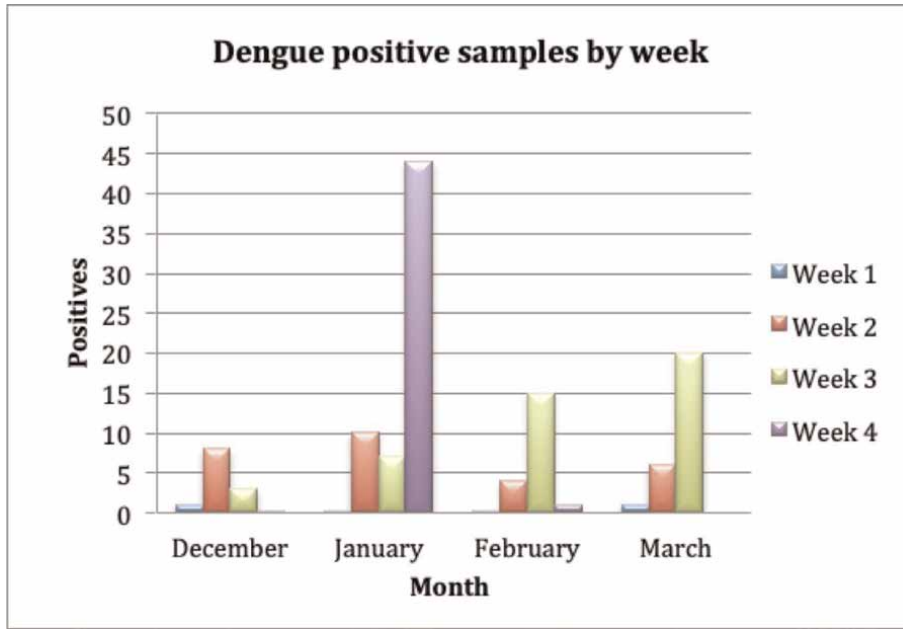

Figure 6.
Shows the weekly positive results from December to the 24th March 2017.

In addition, when we looked at the distribution of dengue cases across the islands, we found a statistically significant concentration of infection on the islands of Emae ($p < 0.00001$), Tongoa ($p < 0.00001$) and Ambae ($p < 0.00001$) compared to the regional average, which was calculated to be 0.416 per 1000 people, suggesting that these islands may possibly harbour animal reservoirs infected with the dengue virus.

9. Conclusion

In summary it has been shown that the pan-family screening approach is a sensitive and specific method for the detection of viral families that contain a large number of diverse pathogens. Viruses will continue to emerge from animal and avian hosts in the future and at present there are very few assays that can detect complex viral families. Coronaviruses are a good example of a family of viruses that have adapted well to human-to-human transmission. In just 20 years three significant pathogens, SARS-CoV-1, MERS-CoV and SARS-CoV-2, have emerged from zoonotic hosts and resulted in two epidemics and one global pandemic which has infected more than 620,000,000 people. It is likely that in the near future a new coronavirus variant will emerge and spill over into the human population resulting in significant morbidity and mortality.

Individual flavivirus and alphaviruses have to date shown different global distribution patterns. Yellow fever is predominately found in African and South America with JEV mainly confined to Asia. It has been suggested that new flaviviruses will continue to emerge or re-emerge into the human population which may cause more serious infections than previously realised as was the case with the recent Zika virus epidemics. Climate change [88] will challenge the current distribution of these

viruses globally as was demonstrated recently with JEV which for the first time was found in Victoria and New South Wales, Australia [89, 90]. The pan-family assays have been tested using insect vectors to screen for flavi- and alphaviruses and preliminary results look promising (John Waitumbi, personal communication) opening the potential of these assays to be used to screen arbovirus vectors for the presence of novel or re-emerging pathogens. One advantage of the current pan-flavivirus/pan-alphavirus/pan-dengue screening test is that the assays can be used in any region worldwide to quickly detect the presence of an unknown arboviral infection and with the boundaries to infection expanding their use is even more urgent.

It would be possible to design unique primer and probe sets that covered the major families of viruses that are pathogenic to the human population. These assays could be multiplexed to produce screening panels that could be used in front line hospitals or sentinel laboratories to screen animal, bats, birds, or vectors such as mosquitoes at regular intervals for emerging viruses. If a sample is positive using the pan-family assay but negative using species specific primers the sample could then be quickly screened by NGS to determine if a novel virus is present. In this way we would be forewarned to the presence of an emerging viral threat.

This simplifies and reduces the costs of broad screening approaches in disease outbreaks or during pathogen surveillance in humans, animal or vectors and importantly has the possibility to identify emerging pathogens without prior sequence knowledge.

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Conflict of interest

DM and JR are paid employees of Genetic Signature the inventors of 3base™ technology.

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Chapter 9

Imaging in Dengue Fever

Rolando Reyna

Abstract

Dengue is a viral disease caused by a flavivirus transmitted by Aedes aegypti mosquitoes in tropical regions but has spread to regions of Europe, subtropical regions, and South America. The clinic is varied, so imaging methods are important before having a positive confirmatory test. Clinically, dengue is a disease that increases vascular permeability with loss of plasma and albumin, causing polyserotis. The most accessible imaging methods in the emergency room are chest radiography and abdominal ultrasound. Chest radiography shows that the most frequent finding is pleural effusion. Abdominal ultrasound has several findings, including thickened gallbladder wall, ascites, and hepatic and splenomegaly. The thickened gallbladder wall is an indicator of disease severity since the more severe the thickening, the more severe the clinical picture. The patient's platelet count is also related to the ultrasound findings, since the lower the platelet count, the more severe is the thickened gallbladder wall. The differential diagnosis of dengue should include other febrile states such as influenza, Zika, Chikungunya, and COVID-19.

Keywords: dengue, abdominal ultrasound, pleural effusion, gallbladder wall thickening, ascites

1. Introduction

Dengue is the most important arboviral infection affecting humans and presents a major challenge for public health services worldwide.

Most infections are asymptomatic or result in only a brief systemic viral illness; a small proportion of patients develop potentially fatal complications.

Although dengue fever disease is mild in most cases and does not progress to severe disease, it can cause many cases in an epidemic form, resulting in overcrowding of health services. Therefore, the ability to recognize cases that progress to severe disease is important.

The World Health Organization classifies dengue into two main categories: dengue with or without warning signs and severe dengue. The secondary classification of dengue with or without warning signs is designed to assist health care professionals in selecting patients for hospital admission for close observation and to minimize the risk of progression to the more severe form of dengue.

The differential diagnosis should be made with febrile states (especially if it is in time of dengue epidemic), such as influenza, Zika, Chikungunya, Hanta Virus (in regions with endemic cases of hanta), and COVID-19.

2. Imaging methods

The most frequently used imaging methods in dengue are chest radiography and abdominal ultrasound, especially in emergency rooms.

The initial evaluation of a patient with dengue is with chest X-ray, and according to the clinical picture and its evolution, other diagnostic methods are requested [1, 2].

Dengue consists of a significant increase in vascular permeability, with loss of plasma and albumin from the intravascular space, causing polyserositis.

Abdominal ultrasound is a widely available imaging technique to study abdominal pain and acute febrile processes. It allows to assess with a high degree of certainty the abdominal findings related to dengue fever, which are thickening of the gallbladder wall, ascites, hepatomegaly and splenomegaly, pericardial effusion, and pleural effusion [2–4].

In chest radiography, pleural effusion is the most frequent finding, which can be unilateral or bilateral, of variable quantity and mainly on the right side. In cases of severe dengue, it may demonstrate the presence of vascular congestion or lead to acute respiratory distress syndrome [4, 5].

3. Imaging findings related to dengue fever

3.1 Gallbladder wall thickening

It is one of the most frequent findings, but it is non-specific since it is found in other viral infections, cholecystitis, liver cirrhosis, and portal hypertension. There are different forms of gallbladder wall thickening that can be observed in ultrasound. These can be lamellar or layered, diffuse, and reticular thickening. Of these forms of thickening, the diffuse thickening is the most frequent form. Lamellar and reticular thickening are observed more frequently in children or young adults. Reticular thickening is more frequent in patients with severe dengue. This type of thickening is usually located at the bottom of the gallbladder **Figures 1** and **2** [4, 5].

3.2 Ascites

Ascites develops with the pathophysiological process of polyserositis, correlating with the severity of the disease. Ascites is detected on physical examination when it exceeds 1000 cc in volume, while ultrasound can demonstrate the existence of scant amounts of peritoneal fluid (approx. 100 cc). Its appearance is usually anechoic and may be of variable quantity. **Figures 3** and **4** [5, 6].

3.3 Pleural effusion

As in ascites, pleural effusion is part of the process of polyserositis, resulting in plasma leakage into the pleural cavity. It is generally an infrequent finding being right or bilateral. Pleural effusion in dengue is one of the markers of severity, but it is mild and self-limiting without the need for intervention. The type of pleural effusion is exudative. **Figures 5** and **6** [4–7].

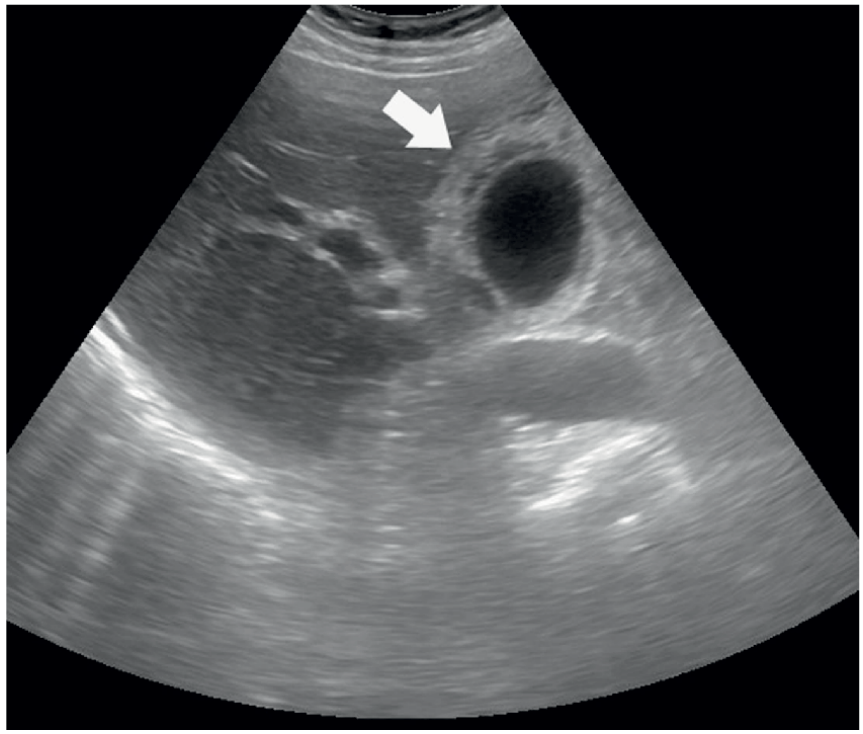

Figure 1.
Abdominal ultrasound axial section. Diffuse gallbladder wall thickening is observed diffusely, (white arrow).

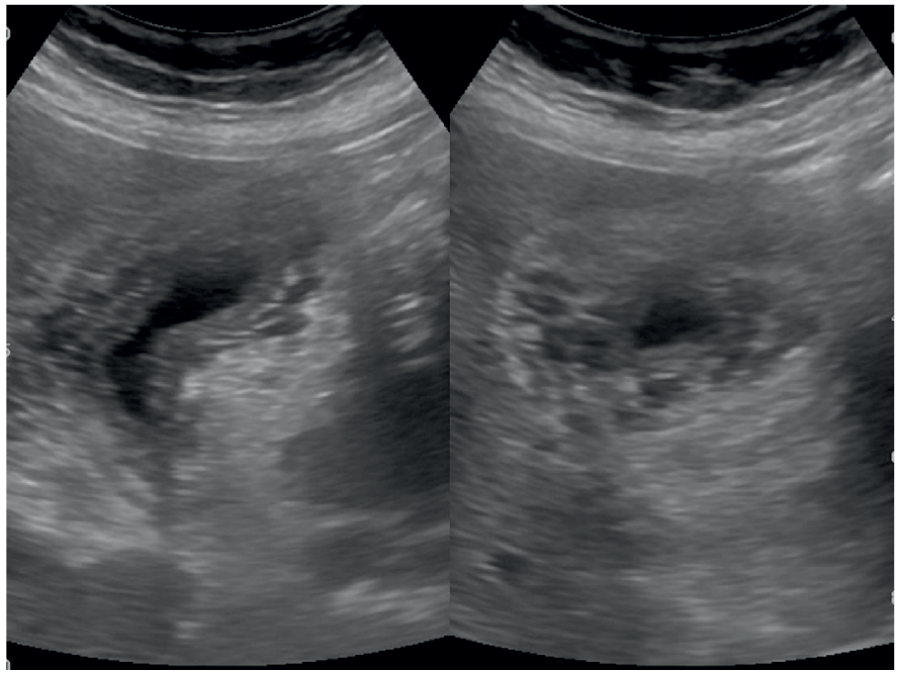

Figure 2.
Abdominal ultrasound. Gallbladder wall thickening with ill-defined lamellar appearance.

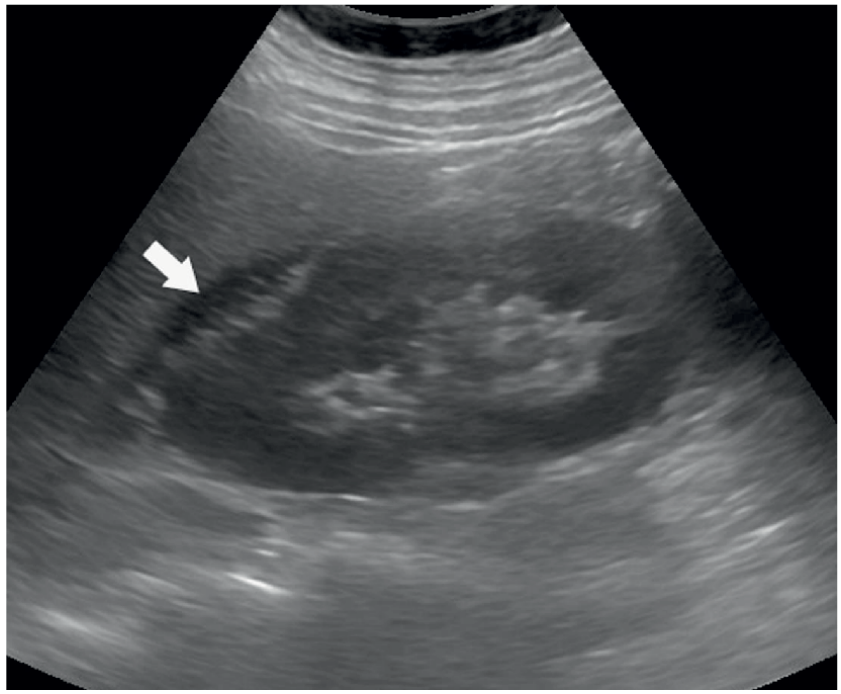

Figure 3.
Abdominal ultrasound. The presence of free fluid around the right kidney is observed at the level of Morrison's fossa (White arrow).

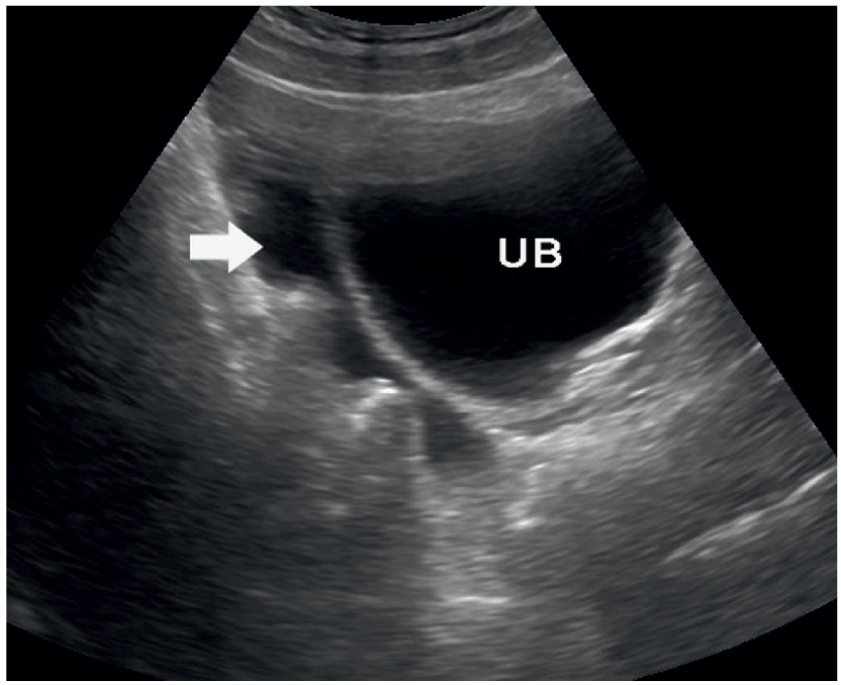

Figure 4.
Free fluid in the pelvic excavation. UB: urinary bladder, (White arrow).

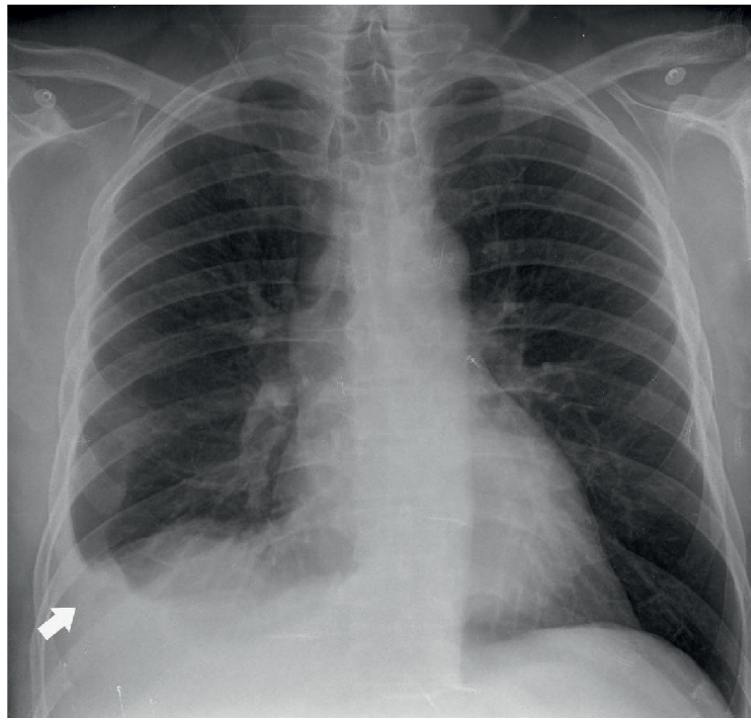

Figure 5.
Chest X-ray. Right costal diaphragmatic angle obliteration due to pleural effusion (arrow).

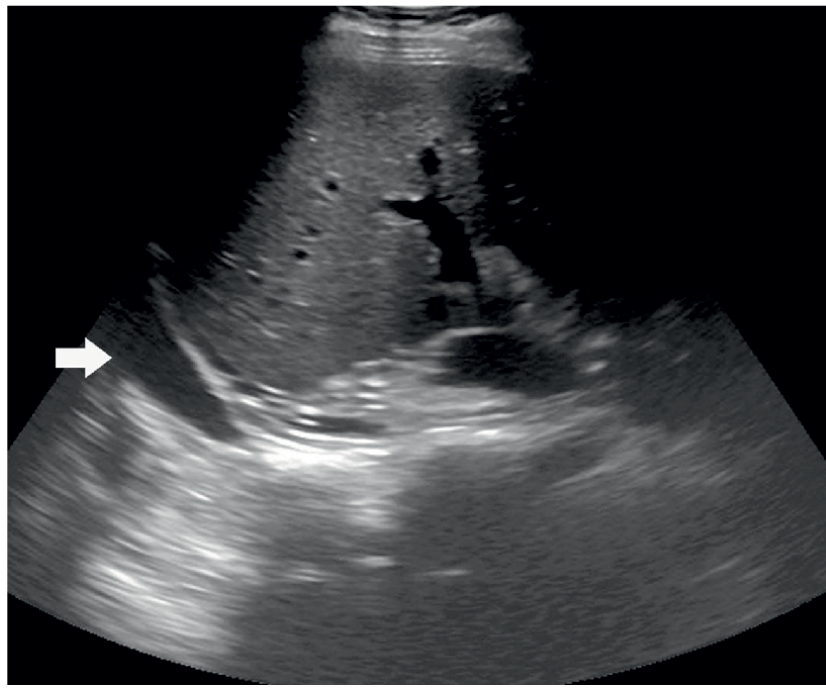

Figure 6.
Abdominal Ultrasound. Transverse hepatic view. Shows simple pleural effusion on the right side (White arrow).

3.4 Hepatomegaly and splenomegaly

Both hepatomegaly and splenomegaly's growth is homogeneous, without focal lesions. In some cases, the liver may present steatosis. Liver growth may be present in up to 30% of cases of dengue fever. Splenomegaly may be present in 14% of cases. **Figure 7** [7].

3.5 Pericardial effusion

It may occur in severe cases after the fifth or seventh day of illness in up to 28% of cases. Its sonographic characteristic is a simple anechoic effusion [7, 8].

There may be a combination of sonographic findings in a patient with a diagnosis of dengue. We can find gallbladder wall thickening with ascites and pleural effusion at any age.

4. Platelet count and imaging findings

Several hematological parameters have been considered as potential predictors, most commonly the platelet count.

The severity of the course of the disease, which is directly linked to the platelet count, can also be assessed by sonography.

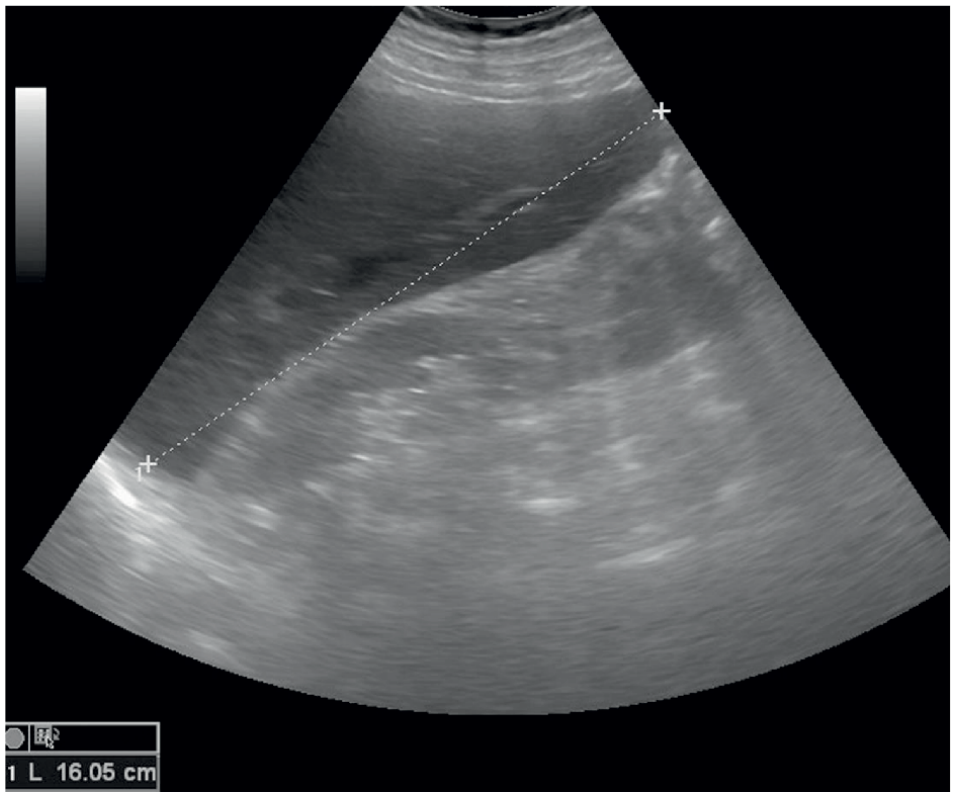

Figure 7.
Abdominal Ultrasound. Hepatic cross-sectional view. Shows mild liver enlargement with homogeneous liver parenchyma.

In patients whose platelet counts are less than 40,000, the most frequent findings are gallbladder wall thickening, ascites, and pleural effusion. With platelet counts between 40,000 and 80,000, the most frequent findings are gallbladder wall thickening and pleural effusion. With platelet counts greater than 80,000, pleural effusion is more frequent followed by gallbladder wall thickening [7].

5. Conclusion

In the clinical context of a patient with suspected dengue fever, findings of gallbladder wall thickening, ascites, pleural effusion, and hepato-splenomegaly strongly favor the diagnosis of dengue fever. An abdominal ultrasound examination can effectively recognize these and guide the clinician to initiate prompt treatment without waiting for serologic results. Ultrasound can also estimate the severity of the disease. The degree of thrombocytopenia shows a direct relationship with abnormal ultrasound findings.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 10

Dengue Virus Gene-Silencing Techniques: A Current Assessment

Samir Casseb, Karla Melo, Carolina Santos and Edna Franco

Abstract

Infection with the Dengue virus (DENV) has become a global threat, affecting approximately 100 nations. There is not a recognized antiviral treatment for dengue at the moment. Therefore, it is crucial to create therapeutic approaches to treat this fatal condition. A critical and successful method of silencing genes, RNA interference breaks down targeted RNA according to its sequence. Over the past ten years, a number of studies have been carried out to determine how well siRNA works to prevent dengue virus replication. CRISPR (clustered regularly interspaced short palindromic repeats) is becoming one of the most effective and widely used tools for RNA and DNA manipulation in numerous organisms. In our review, we describe and discuss the use of these technologies to comprehend and treat DENV-related infections.

Keywords: dengue, CRISPR, RNAi, genetic engineering, siRNA

1. Introduction

Dengue is the most dangerous virus spread by mosquitoes, and any of the four DENV serotypes (DENV-1 to DENV-4) can cause it. A DENV infection can cause a broad spectrum of clinical symptoms, ranging from a mild flu-like condition known as dengue fever (DF) to the potentially fatal dengue shock syndrome (DSS) [1].

Approximately half of the global population is at risk for Dengue fever, and the mosquito-borne virus is the leading cause of death in certain Latin American and Asian nations. Nevertheless, despite the rapid increase in cases and decades of drug development efforts, there is no specific treatment and only one vaccine with a limited application [2].

Symptoms of DF include fever, nausea, vomiting, rash, and aches and pains; however, in DSS, severe hemorrhage and shock can develop, and if left untreated, the fatality rate can reach 20%. Previously, the World Health Organization (WHO) classified dengue disease states as undifferentiated fever, dengue fever, and dengue hemorrhagic fever (DHF) [3].

The categorization of DHF was revised into four levels of severity, with grades III and IV being classified as DSS. However, in 2009, the WHO updated its case categorization method, discontinuing the previous categories of probable Dengue, Dengue with unexpected symptoms, Dengue with warning symptoms, and severe Dengue. Currently, the focus is on understanding DENV's biology, epidemiology, and

transmission characteristics, including circulating serotypes and genotypes, DENV-specific immune responses, illness etiology, improved diagnostic tools, therapies, and vaccine development [2, 3].

There is no antiviral treatment for dengue fever, and the only approved vaccine, Dengvaxia from Sanofi, can be dangerous. Dengvaxia can reduce the severity of Dengue fever in previously infected individuals. However, Dengvaxia may increase the risk of severe Dengue in uninfected individuals [4].

Experts say that the development of vaccines and antivirals has been slowed down by poorly coordinated clinical trials, problems with animal models and lab tests, and a complicated and constantly changing virus. Experts say that manufacturers could make progress on this disease if they simplified the endpoints for symptoms in clinical trials and used less common designs like platform trials and human challenge studies [5].

In this way, scientists worldwide have been working hard to find treatments and ways to avoid getting sick. In the search for treatments to stop the spread of DENV, new technologies like RNA interference (RNAi) and CRISPR have become more popular.

2. Dengue treatment technologies

As previously stated, diverse dengue treatment technologies are currently being developed. Our text will elaborate on RNAi and CRISPR, two technologies that are getting more and more interesting in this field.

2.1 RNAi

Post-transcriptional gene silencing (PTGS) is observed in many species, including plants, fungi, and animals. RNA interference (RNAi), an ancient defense mechanism, is the common denominator [6].

When put into cells, long dsRNA can efficiently and precisely lead to the degradation of cognate mRNAs in a way that depends on the gene. This powerful technology has been used to change how genes are expressed, determine how signals are sent, and determine what genes do on a whole-genome scale [7].

Researchers worldwide have used RNA interference (RNAi) for basic research. They are currently making drugs based on RNAi to prevent and treat viral infections, tumors, and metabolic disorders in humans [8].

Although there have been significant improvements in the treatment of viral diseases, current medications and vaccines are still limited by a variety of issues, including toxicity, complexity, cost, and resistance. Eukaryotic get a defense mechanism called RNAi that helps them avoid getting infected by viruses [9].

Viral mRNA is sent to cellular enzymes to be broken down, which can stop the production of crucial viral proteins. Human cells can now be protected from viruses that cause disease thanks to new technology [10].

2.1.1 Machinery of RNAi

Through biochemical and genetic research, scientists have discovered how dsRNA causes the breakdown of target messenger RNA at the molecular level. RNA interference involves the initiation and effector steps [9].

Dicer, a member of the RNase III family of ATP-dependent ribonucleases, binds to long dsRNA (introduced directly or via a transgene or virus) with high affinity and cleaves it into small interfering RNA (siRNA) duplexes. An N-terminal DEXH-box RNA helicase domain, a domain with an unknown function (DUF283), a PAZ domain, two RIII domains, and a dsRNA-binding domain are all common features of dicer enzymes (dsRBD). In order to create siRNAs or microRNAs (miRNAs), the dicer can cut stem-loop precursors from dsRNA [11].

siRNAs are dsRNA duplexes with 21–23 nucleotides, 2-nt 3′ overhangs, a 5′-monophosphate, and a 3′-hydroxyl group. During the "effector" (RISC) step, siRNA duplexes are incorporated into the RNA-induced silencing complex (RISC). The phosphorylation of the 5′-terminus of siRNA is required for entry into RISC. A helicase domain of RISC binds to one end of the duplex and unwinds it ATP-dependently [12].

The thermodynamic stability of the initial few base pairs of siRNA can affect the proportion of RISC containing antisense or sense siRNA strands. Dicer with R2D2 (Dcr-2-associated protein) binds siRNA and assists with its loading onto RISC. The active RISC then identifies the homologous transcript via base-pairing interactions and cleaves the mRNA between the 10th and 11th nucleotides of the 5′ end of the siRNAs [13, 14].

Animals make these short RNA species using Dicer to cut long (70 nt) endogenous precursors with an imperfect hairpin RNA structure into short RNA species. Mature miRNAs stop translation by partially matching their bases to the 5′ or 3′ ends of mRNAs. A miRNA that is completely complementary to its target mRNA (endogenous siRNA) can cause the target mRNA to be broken down [15, 16].

Furthermore, it is likely that many other proteins, such as eukaryotic translation initiation factor 2C2 (eIF2C2) and Argonaute proteins, work in both pathways. Argonaute proteins are the essential RISC components. With two distinct domains, PAZ and PIWI, they are evolutionarily conserved. The PIWI domain is exclusive to Argonautes, whereas the PAZ domain is shared with proteins 21 from the Dicer family [13, 17].

2.1.2 Silencing mechanisms of RNAi

The mRNA targets multiple siRNA sequences, and long dsRNA effectively stops the gene from being expressed. Virus-infected cells always produce dsRNA, but viruses can evade a severe cellular response. The dsRNA binds to dsRNA-binding proteins (dsRBPs), which have been shown to stop RNA interference (RNAi) and block the effects of interferon (IFN). Recent research has shown that 21-nucleotide siRNAs cannot cause mammalian cells to make interferon. Since siRNAs can stop viruses from spreading, more and more scientists are becoming interested in this field [16].

It has been shown that siRNA molecules can stop a virus from spreading by sending viral mRNA to be broken down. Compared to other conventional medications, siRNA has numerous advantages. Because sequence-specific target mRNA and complementary siRNA make it much easier and more flexible to choose target sites, siRNAs can stop mRNA from doing its job by going after different parts of the target mRNA for a given mRNA molecule. Second, to silence a gene, a substoichiometric amount of siRNA is enough to reduce homologous mRNA by a lot within 24 hours [18].

Third, siRNAs can cause cognate mRNA to break down in the cells of different species. Scientists are working on siRNA delivery systems that will make it easier for

siRNA to get into the cells of almost all organs. Fourthly, siRNAs appear to have no negative effect on cell control mechanisms. The length of the siRNA and how similar it is to the target region of the cognate transcription make sure that only the desired transcript will be destroyed. siRNAs lacking suitable targets appear to be inactive within cells. The best thing about RNAi as a way to fight viruses is that it is very specific and does not have any bad side effects.

Fifthly, siRNAs can effectively silence genes. Using plasmid and viral vectors, siRNAs can exhibit their long-lasting biological effects. The siRNAs made in vivo or in vitro and then put into cultured cells or animals may silence messenger RNA (mRNA) molecules based on their sequence. Since proof-of-concept studies showed that siRNAs work, they have become a popular alternative therapy [19].

2.1.3 RNAi and DENV

RNA interference is an exciting field of functional genomics that can silence viral genes. This virus-fighting system, found in many eukaryotes, could be used to treat flavivirus infections in hosts. However, RNA interference against flaviviruses has received scant research [20, 21].

RNAi has been utilized against multiple human pathogens, such as human immunodeficiency virus type 1, hepatitis C virus, hepatitis B virus, poliovirus, influenza virus A, and DENV. In the cytoplasm, the ssRNA genomes of these viruses are visible and could be used as RNAi targets. Between viral RNA uncoating and viral replication, this occurs [22].

Certain mosquitoes are capable vectors of arthropod-borne viruses (arboviruses), while others are not. It has been established that Aedes species possess a Rnai pathway. The first piece of evidence is that recombinant Sindbis viruses expressing an RNA fragment from a genetically unrelated dengue-2 virus (DENV-2) inhibit DENV-2 replication in *Aedes aegypti* mosquitoes in a manner analogous to how plants shut down genes [6].

The second evidence is that the replication of the homologous virus is stopped when dsRNA or siRNA made from the arbovirus genome is put into C6/36 (*Aedes albopictus*) cells. The third evidence is that virus-resistant C6/36 cells were made from DENV-2-specific hairpin RNA copied from a plasmid. These things show that RNA interference is present in *Aedes* species, just like in plants and other animals [23].

Both innate and adaptive immune responses highly influence the DENV infection, but little is known about the innate immune response of the mosquito vector *A. aegypti* to arbovirus infection. DENV-2 does not completely evade RNA interference, as silencing the expression of dcr2, r2d2, or ago2 genes increases virus replication in vectors and shortens the extrinsic incubation period for viral transmission. Sánchez-Vargas and his team showed that RNA interference is a key factor in controlling mosquito infections [24].

Dendritic cells (DC) infected with AAV-siRNA demonstrated a dose-dependent reduction in dengue infection. DCs treated with AAV-siRNA were also protected from dengue-induced apoptosis. Thus, AAV-mediated siRNA delivery can reduce dengue infection and replication in humans. Through extensive siRNA screening, more than 100 proteins of host factors involved in DENV replication have been identified. In drug design, these host factors serve as drug targets. Host factors (proteases, glucosidases, other) have yet to be identified via siRNA screening. Also, these studies could not find genes for natural immunity that protect against DENV infection. The biggest problem is getting siRNA to patients; a good way to do that has yet to be found [25].

The fact that DENV-2-derived siRNA was found in RNA extracts from the midguts of Carb77 and that the resistance phenotype was lost when the RNAi pathway was blocked [26] showed that an RNAi response caused DENV-2 resistance. C6/36 cells transfected with siRNA against the dengue PreM gene were then attacked by the DENV1 virus [25].

After seven days, the number of transfected cells that were still alive increased by 2.26 times, while the amount of virus RNA dropped by 97.54 percent. This finding provides evidence that siRNA inhibits dengue replication effectively [27]. Mukherjee et al. [28] showed that DENV can replicate in Drosophila S2 cells and that the RNAi pathway controls DENV replication. The downregulation of HSP60 in infected cells reduced viral load, RNA copy number, and IFN concentration [29].

High levels of HSP60 in infected cells make it easier for viruses to multiply and could be a target for treating dengue infection. RNAi, plasmid transfection, and inducible vectors can temporarily turn off genes' effects. siRNA is extremely specific for target RNA. Therefore, siRNA is important for discovering and understanding gene function [29, 30].

Using siRNA to silence the attachment receptor and clathrin-mediated endocytosis, it is possible to lower the amount of virus in like this using siRNA to stop the attachment receptor and clathrin-mediated endocytosis, the amount of virus in the body can be lowered. Thus, preventing the progression of dengue fever to more severe forms (DHF/DSS) [31].

Importantly DENV infection identified key cellular genes involved in endocytosis and cytoskeletal dynamics. siRNA targeting genes involved in clathrin-mediated endocytosis prevented DENV entry into Huh7 cells [32]. Villegas-Rosales et al. [26] recently found that three siRNAs that target NS4B and NS5 sequences can silence four DENV genome serotypes.

Combining siRNA and endogenous RNAi processing machinery can prevent severe dengue infection. DC-3 siRNA is a new way to fight against different serotypes of Dengue, so it can help develop new treatment plans [33].

Korrapati et al. [34] used a human adenovirus type 5 vector that could not replicate to target conserved viral genome sites with short-hairpin RNA. This short-hairpin RNA grows into the corresponding siRNA and stops all four dengue serotypes from making antigens and more viruses.

These studies and their clear results show that RNA interference prevents DENV from replicating in cell cultures and animal models [35].

2.2 CRISPR

This adaptive immune response protects bacteria and archaea from bacteriophages and plasmids. CRISPR-Cas immunity is mediated by crRNA and an endonuclease Cas that targets genetic elements. The mode of action includes three distinct phases: acquisition, expression, and interference. In the acquisition step, foreign nucleic acids are added in a specific order as new CRISPR spacers to a CRISPR array made up of repeat sequences. This creates a memory of the genetic elements outside the cell [36–38].

The CRISPR locus is turned into a pre-CRISPR RNA transcript (pre-crRNA) during the expression step. This pre-crRNA is then changed into a mature crRNA that has some CRISPR spacer sequences joined to some CRISPR repeats. A trans-activating RNA (tracrRNA) is also made by the CRISPR locus. Its repeat regions match those of the crRNA transcripts. In addition to the CRISPR array, the CRISPR

locus can code for one or more Cas nucleases, such as Cas9. During the interference phase, the repeat region sequences that match each other bind to make a hybrid of crRNA and tracrRNA. This RNA hybrid tells the Cas nuclease to go after complementary DNA sequences. This allows invading genetic elements to be found and cut out [39, 40].

Most CRISPR effector proteins depend on a protospacer-adjacent motif (PAM) in the targeted nucleic acid, like NGG for Cas9. The PAM is essential for self-DNA recognition, cleavage, and differentiation from non-self DNA [41].

For Cas9, perfect complementarity will cause the endonuclease to change shape, leading to a structure that can cut DNA. The protein and RNA parts of Streptococcus pyogenes's class 2 CRISPR system have been changed to work in eukaryotic cells, like human cells [42].

Mammalian cells send Cas9 to the nucleus by joining it to a nuclear localization signal (NLS) that works best with human codons. To make single-guide RNAs (sgRNAs) for editing the genome that looks like the natural crRNA–tracrRNA hybrid, crRNA-like sequences are fused to a partial tracrRNA using a synthetic stem-loop [43].

2.2.1 Gain-of-function approaches

Strategies that use the ectopic overexpression of genes have helped find cell surface receptors needed for viruses to get into cells and host factors that stop viruses from getting into cells. An infection-resistant cell line is often transduced with a complementary DNA library (cDNA library) made from an infection-permissive cell type to find entry receptors. In a cDNA library made from hepatocellular carcinoma cells and a non-permissive cell line, claudin 1 (CLDN1) and occludin (OCLN) were found to be HCV entry receptors [44, 45].

In addition to identifying receptors, an independent expression screen revealed that SEC14-like protein 2 (SEC14L2), a cytosolic lipid-binding protein, promotes the replication of clinical strains of HCV20. Also, proteins important for the immune system's natural defenses against DNA and RNA viruses were found using a library of about 380 interferon-stimulated genes (ISGs) [46–48].

In addition to these screens, full cDNA libraries with all annotated ORFs from humans have been cloned into lentiviral expression vectors. This has led to the creation of an expression vector library, which will likely make the gain of function screens more useful for studying the interactions between a host and a pathogen [49, 50].

2.2.2 Function loss genetic analyses

Screens for loss of function rely on the stable knockdown or knockout of genes. Initial RNA interference-based approaches have yielded valuable insights into virus-host relationships [50].

In contrast to RNAi, which only stops some genes from being expressed, recent technological advances have made it possible to stop all genes from being expressed. One way is to use insertion mutagenesis to change genes in haploid cell lines in culture. This is called "haploid genetic screening." Retroviral gene traps with a splice acceptor site, for example, can become part of the host genome and cause truncated mRNA transcripts to be made. Completely turning off the expression of a gene can have big effects on viral replication and help figure out which parts of the host are most

important for viral infection. Using insertion mutagenesis in haploid cells, researchers have found the essential receptors for many viruses, like Ebola and Lassa [51, 52].

As receptors, both viruses utilize abundant lysosomal proteins. The interaction between the Ebola virus glycoprotein and its receptor Niemann–Pick C1 protein (NPC1) is set off by cathepsin cleavage. In contrast, the interaction between the Lassa virus glycoprotein and its receptor lysosome-associated membrane glycoprotein 1 (LAMP1) is set off by acidification of the endosome. Subsequent structural studies determined the viral glycoprotein and NPC1 binding interface. During the 2013–2016 Ebola epidemic, several mutations occurred in the host-binding site of the viral glycoprotein [53–56].

These changes made the virus more infectious in cells from primates but not in cells from rodents. This implies that they aided the virus's adaptation and spread in humans. Haplotypic genetic screens helped find a cellular phospholipase that lets viruses get around an antiviral restriction mechanism that works against many *picornaviruses* [57].

Recently, a haploid screen found a protein-based receptor that allows multiple different serotypes of adeno-associated virus (AAV) to enter cells. This may change how AAV is used as a vector for gene therapy. Loss of function screens is a good way to find out which host factors are necessary for viral replication, as shown by these and other studies [58].

2.2.3 Insights from CRISPR-CAS screens

CRISPR-Cas screens have a great potential for identifying host factors essential for viral pathogenesis, which could lead to developing new antivirals. CRISPR-Cas screens have been used to study several viruses [59].

CRISPR-Cas screens could find host factors essential for viral pathogenesis, which could lead to developing new antivirals [60].

Using CRISPR-Cas screens, the cotranslational and posttranslational insertion of several membrane-spanning hydrophobic helices and polyprotein cleavage by a viral protease and several host proteases into the mature viral proteins have been studied. Even though these processes are known, not enough is known about the involved host proteins [61, 62].

Using DENV, different CRISPR-Cas screens have each found several ER proteins needed for the virus to spread. A lot of these proteins are needed for the endoplasmic reticulum (ER) to do its important job of making membrane and secretory proteins [63].

The identified proteins have been implicated specifically in N-linked glycosylation, ERAD, and signal peptide insertion and processing. Notably, these proteins were identified in duplicate screens conducted in the same lab as well as independent screens conducted in separate labs using distinct cell lines and virus strains. There was also substantial overlap between the results of haploid genetic testing. This technology's remarkable reproducibility is a major advantage [64].

Furthermore, CRISPR-Cas technology is a reliable way to test candidate genes and figure out how gene knockouts affect a virus copies itself. Gene knockouts differ from knockdown methods like RNA interference (RNAi) because they are permanent and do not lead to different levels of depletion. This lets people use quantitative tests for virus replication, like quantitative PCR, immunostaining, or plaque assays, to compare genes accurately. When the most enriched host factors were taken out of the screens, flavivirus replication dropped by 100–10,000,000. This shows that pooled sgRNA screens could be used to find host factors needed for virus replication [65].

CRISPR-Cas knockout cells can also be used to understand the molecular basis of knockout phenotypes and find out which stage of a virus's life cycle the host factor is involved. For instance, it was discovered that the OST complex is required for viral RNA synthesis but not for viral entry and translation [63].

The OST complex glycosylates newly synthesized proteins via N-linked glycosylation. In mammalian cells, there are two different OST multiprotein complexes. Each comprises a catalytic subunit (one of two paralogs, STT3A or STT3B) and accessory subunits [66].

DENV replication needs both isoforms, as either knocking out STT3A or STT3B stopped DENV replication completely. Other *flaviviruses* that are spread by mosquitoes, like ZIKV, only use the STT3A isoform for viral RNA replication. This strongly suggests that the virus and the host interact differently. Inactive mutant proteins were able to bring DENV replication back to the knockout cells. This proves that the OST complex plays a role in DENV replication that was not expected. Multiple viral proteins that are not structural but are part of the RNA synthesis complex at ER61 were found to bind to the OST complex. This suggests that the OST complex is a framework to help create a DENV RNA replication complex that works [67, 68].

SEC61A1 and SEC63, which form the translocon channel in the ER membrane; the translocon-associated protein (TRAP) complex, which stimulates cotranslational translocation of polypeptides into the ER73; and the signal peptidase complex, which cuts signal peptides in the ER lumen are also essential for flavivirus replication. Multiple *flaviviruses* exhibited severe polyprotein cleavage deficiencies when a subset of signal peptidase complex subunits (SPCSs) was absent. Particularly, the cleavage of the structural proteins prM and E from the polyprotein was impaired, resulting in significant defects in virus particle release [69, 70].

Other host factors essential for flavivirus replication include SEC61A1 and SEC63, which form the translocon channel in the ER membrane; the translocon-associated protein (TRAP) complex, which stimulates cotranslational translocation of polypeptides into the ER73; and the signal peptidase complex, which cuts signal peptides in the ER lumen. Multiple flaviviruses exhibited severe polyprotein cleavage deficiencies when a subset of signal peptidase complex subunits (SPCSs) was absent. In particular, separating the structural proteins prM and E from the polyprotein was hard for the virus particles to get out of the cell [71].

It is important to know that genetic screenings of WNV and DENV have not found a specific receptor for viruses to enter host cells. This is not the case with many other viruses, such as Ebola. This is probably because there is more than one way for a virus to get into a cell. If a virus receptor is knocked out, the cell is still open to infection in a different way. Indeed, numerous DENV receptors have been identified. Nevertheless, CRISPR-Cas screens have contributed to our understanding of flavivirus biology by revealing the central role of ER complexes in flavivirus infection promotion [51, 71].

2.2.4 CRISPR-CAS antiviral strategies

The CRISPR-Cas technology could be used to prevent and treat diseases by going after viruses and the things that spread them. Vector control has been used to stop the spread of viruses carried by vectors, like ZIKV, DENV, and yellow fever [72].

Using CRISPR-Cas tools, scientists have made gene drives that could reduce the number of mosquitoes. CRISPR-Cas technology could also be used to treat HIV, HBV, HCV, and the herpes simplex virus, which do not go away on their own. HBV

covalently closed circular DNA (cccDNA), a sign of a persistent HBV infection, has been successfully targeted in cell cultures and animal models [73–75].

Additionally, CRISPR-Cas screens can be utilized to determine the mechanism of action of antivirals. For example, CRISPR-Cas and short hairpin RNA (shRNA) screens were used to determine how the antiviral drug GSK983 works. This drug may stop a wide range of RNA and DNA viruses. By stopping the enzyme dihydroorotate dehydrogenase from making pyrimidine in cells and lowering the number of nucleotides inside cells, which are needed for viral nucleic acid synthesis, GSK983 was found to stop viruses from spreading [76, 77].

3. Conclusions

Technologies like CRISPR and RNAi have become important ways to learn more about how viruses, like DENV, cause infections.

In addition, it is noteworthy that both CRISPR and RNAi have emerged as viable alternatives for treating viral infections and managing *aedes* vectors.

The new information we get from these technologies will be significant for a better understanding of how viruses replicate and interact with their hosts.

Conflict of interest

The authors declare no conflict of interest.

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Section 5

Management Strategies

Chapter 11

Network Formation and Analysis of Dengue Complex Network

Hafiz Abid Mahmood Malik

Abstract

Several efforts have been made and are constantly being made to keep the *Aedes aegypti* virus under control. Numerous scholars are involved in the study of medicine, while others are working in computer science and mathematics to model the spread of this disease. This study will help to comprehend how this epidemic sickness behaves. A complex network has been established from the complex dengue phenomenon. We have evaluated dengue network topology by pondering scale-free network properties. The network's resilience in tracking the dengue epidemic is measured by systematically removing nodes and links. The primary hubs of this network are emphasized, and the vulnerability of the network structure has been examined through an in-depth investigation of the dengue virus's spreading behavior. Understanding the intricate web of dengue outbreaks relies heavily on geographic representation. The applied method on the dengue epidemic network and the results will be added as scientific additions to the literature on complex networks. Different network analysis metrics have been applied (closeness centrality, betweenness centrality, eigenvector centrality, network density), and the network's stability has been evaluated. This network is extremely vulnerable to targeted attacks; results showed that after removing 8% of focal hubs, 34% of the network is destroyed.

Keywords: vector-borne disease, robustness, dengue vector, scale-free network, complex network metrics

1. Introduction

Most dengue fever cases have been reported in tropical and subtropical areas. However, reports of its spread to numerous other regions, including Europe, have increased significantly during the past decade. There are around 2.5 billion people who could get dengue fever (DF) or dengue hemorrhagic fever (DHF) [1, 2]. In January 2022, the WHO estimated that there might be between 100 and 400 million new dengue cases worldwide yearly. An estimated 3.9 billion people live in these areas where dengue fever arises (**Figure 1**) [1, 2].

The mosquito is the vector for the dengue virus. Bite transmission occurs mostly due to the *A. aegypti* and *Aedes albopictus* species of mosquitoes, both of which are considered the carrier of the disease [2, 3]. *A. aegypti* is a small, dark mosquito with a silvery white pattern of scales on its body and white bands that may be identified on

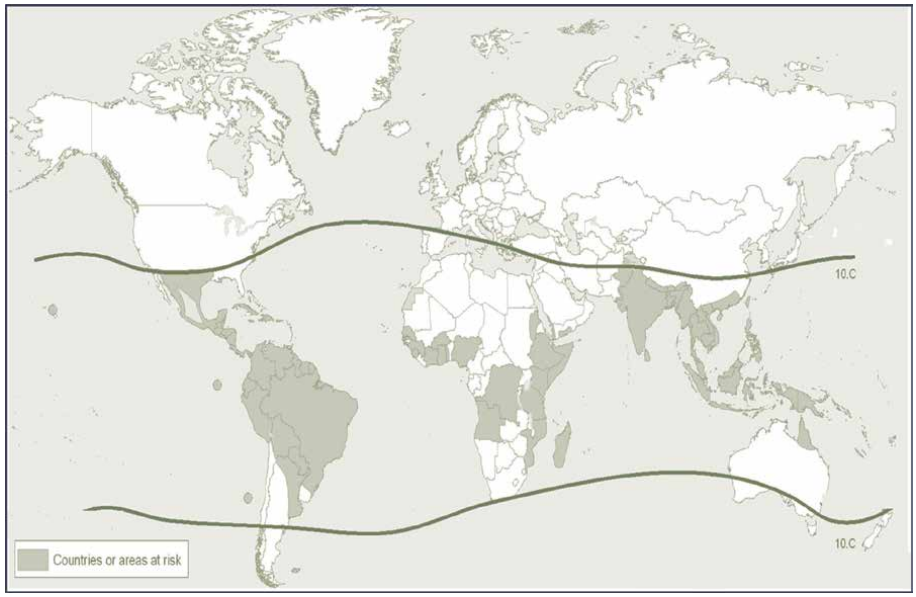

Figure 1.
World map showing locations with a high risk of dengue fever.

Figure 2.
*Dengue vector (*A. aegypti*).*

its legs (**Figure 2**). Most people get bitten by this urban mosquito during dawn and evening [4, 5].

1.1 Dengue virus outbreak

The *A. aegypti* mosquito can spread the dengue virus to humans through a bite. Mostly female *A. aegypti* mosquitoes have been found to carry the dengue virus, which means that females of this type of mosquito may be the superspreader of the disease [4, 6–8]. Female *A. aegypti* have a lifespan of 12–56 days (mean: 34 days) [9, 10]. In order to produce offspring, female *A. aegypti* mosquitoes mate with males and produce offspring by egg-laying; in this way, a male can carry this virus, too. If *A. aegypti* bites any person and that person acquires the dengue virus (DENV), they also become the source of DENV. **Figure 3** [11] depicts the transmission of DENV from an infected

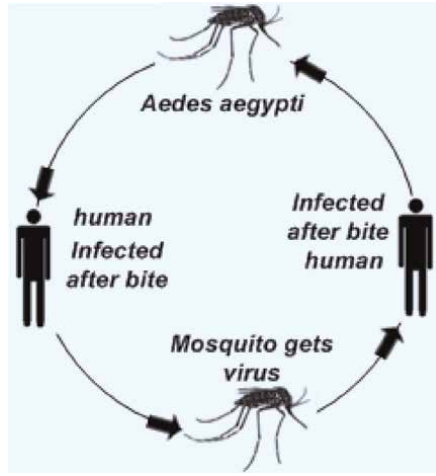

Figure 3.
Dengue spread.

individual to another mosquito (not of the *A. aegypti* species) via a mosquito bite [3, 5]. In addition, dengue symptoms often develop 4–14 days after a mosquito bite. Specifically, the dengue virus's DENV-1, DENV-2, DENV-3, and DENV-4 serotypes have been identified [5, 6, 12]. The infected person's blood can be tested for any of these dengue serotypes. Any individual infected by one of these serotypes is immune to future infection by the same serotype [3, 6, 13].

2. Modeling the dengue epidemic as a two-mode network problem

The structure of some real-world datasets is naturally bipartite. A key characteristic of this sort of network is that it allows for the partitioning of nodes into two groups (primary and secondary) and the creation of linkages exclusively between nodes in the two groups. To define a bipartite graph, we use the triplet $G = (\top, \perp, E)$, where $\top$ is the set of vertices, $\perp$ is the set of top edges, E is the list of bottom edges, and $E \subseteq \top \times \perp$. Whereas in traditional graphs, links usually go from one group of nodes to another, in this case, the nodes are in two separate but intersecting sets. If two nodes (of $\perp$) in G have at least one neighbor (in $\top$), then they are connected in the $\perp -$ projection, which is the graph $G\perp = (\perp, E, \perp)$. In **Figure 4**, a–d depict the primary set of nodes in the two-mode network, whereas 1–5 represent the secondary set [14].

This study uses a dataset of weekly dengue cases from various nodes (locales) in Selangor, Malaysia, to formalize the epidemic problem as a two-mode network. This

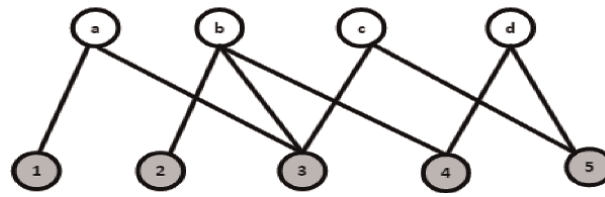

Figure 4.
Two-mode networks, illustrated with an example.

network's primary set of nodes is "localities," whereas the second set is the "number of infected patients in weeks." Two nodes are linked in this network if they have the same number of infected cases within the same week, with the number of cases serving as the link weight. The weighted two-mode network is shown here in **Figure 5**. In Selangor, Malaysia, the weeks are labeled W1, W2, … , W52, and the locations are denoted by the letters PL1, … , GL1, … , and HLL1, … [14, 15].

Limited network analysis metrics can be used for the original, unaltered version of two-mode networks, which is challenging to perform an in-depth analysis [16]. In order to study these kinds of two-way networks, it is common practice first to transform them into a one-mode network.

All of the nodes in a two-mode network are linked by the fortuitous occurrence of weeks, thereby converting the network to a single mode. The actual two-mode network is depicted in **Figure 5**, whereas the one-mode projection is shown in **Figure 6**.

A white node represents the locality in Selangor, while a gray node symbolizes the number of weeks; W1 denotes the first week, W2 the second, W3 the third, W4 the fourth, and W5 the fifth.

Projection is commonly used to transform two-mode networks into single-mode ones [16–18]. Here, we use three different projection techniques—Binary, Sum, and Weighted Newman—to turn a two-mode network into a single-mode one. Based on outcomes, it is determined that the Newman technique is better suited to the dataset under consideration.

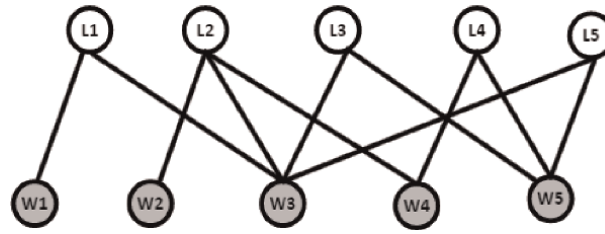

Figure 5.
A simulation based on an actual dataset.

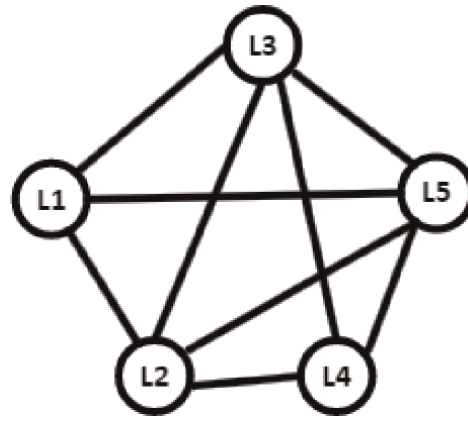

Figure 6.
The two-mode dengue network is transformed into a one-mode from a geographic perspective, represented in ***Figure 5****.*

Since many real-world networks have weighted information in their linkages, Newman's approach does not account for this fact adequately. The Weighted Newman technique is a generalization of Newman's approach that Opsahl suggests [17–19]. He claims that the weight can be expressed mathematically as Eq. (1).

$$w_{ij} = \sum_{p} \frac{w_{ip}}{N_p - 1} \tag{1}$$

The link from node *i* to the co-occurrence has a weight of *wip*, where *wij* is the weight between *i* and *j*.

2.1 Dengue epidemic: A network analysis

Network visualization and analyses of one and two-mode degrees, weighted degree, density, closeness, betweenness, and eigenvector centrality measures are used in this study to examine the dengue outbreak. Primary nodes in the network analysis are the places, whereas secondary nodes are the weeks. As a means of analyzing the predicted results of weighted Newman algorithms, above mentioned network metrics are used.

Figure 7 is a graph depicting the degree centrality of all nodes in Selangor, Malaysia. All of Selangor's dengue hotspots are plotted along the x-axis. Weighted Newman projection using the centrality metric has been utilized for this purpose. Selangor dengue network nodes with the highest centrality are PL216, PL31, PL137, PL134, HLL161, PL54, HLL84, HLL117, and HLL115, which indicates they have a lot of ties to other nodes. Here, the granularity of these vertices can be observed [15, 20].

From the perspective of degree analysis, the simple degree measure has less relevance in this network and is the crude measure. The binary method does not produce satisfactory outcomes when considering the strength of nodes (weighted degree). With the binary approach, it's clear that the degree and strength of a single mode are identical. The weighted Newman technique (**Figure 8**) produced the out-strength of nodes, which may be read as the overall number of dengue cases

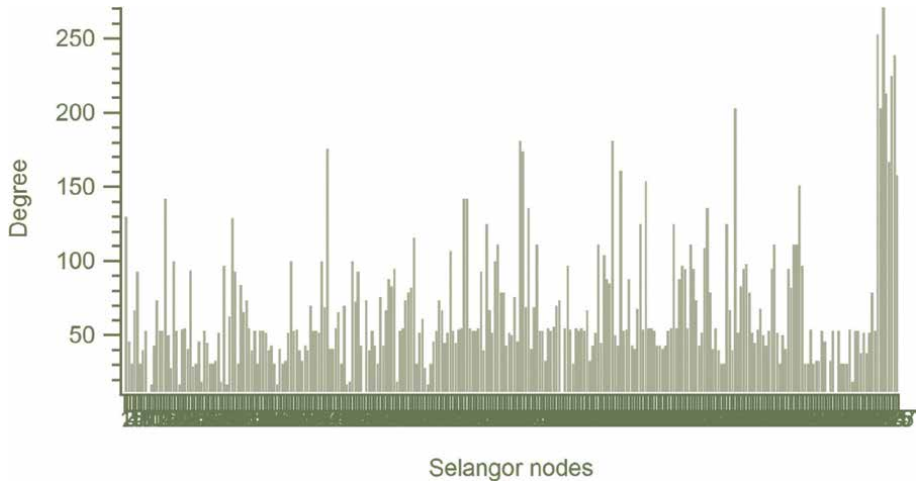

Figure 7.
The weighted Newman degree of nodes.

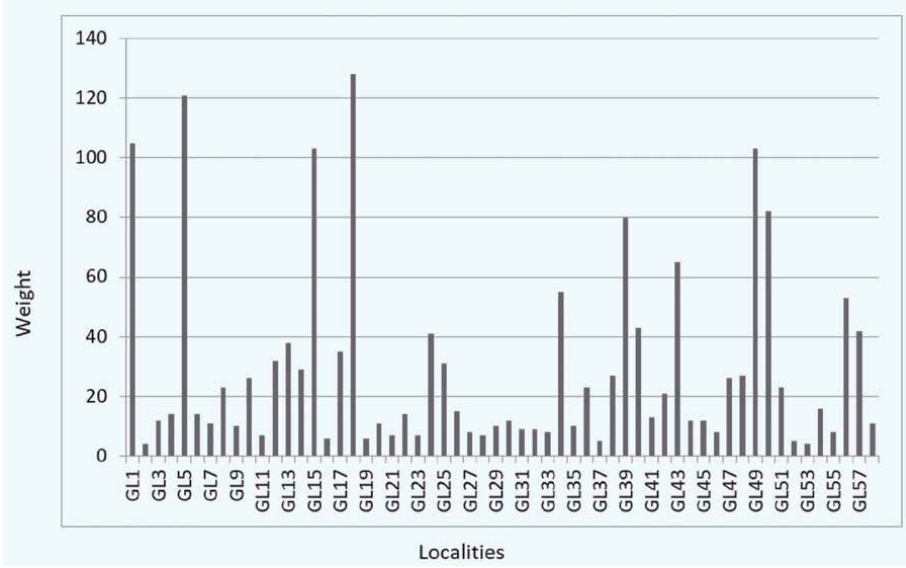

Figure 8.
Gombak network node strengths calculated with the weighted Newman approach.

reported in certain nodes [21]. On the x-axis, 58 dengue-affected nodes in the Gombak district are illustrated, while the y-axis depicts the strength of these nodes. The strongest network strength was seen at node GL18, corresponding to the highest reported node total of dengue cases (128 cases).

Dengue fever cases were also the second most numerous in GL5, with a total of 121 being reported within the specified time frame. There were four confirmed dengue infections in GL2 and none in GL53. The strength of the two-mode projection and one-mode weighted Newman projection method is the same, suggesting that weighted Newman projection generated more suitable outcomes than Sum and Binary projections.

Figure 9 is a graph displaying the measured strength of all Selangor nodes. The x-axis shows the total number of dengue hotspots in Selangor (across six districts), while the y-axis displays their relative strength. The weighted Newman projection method was utilized to evaluate the node strength. The Selangor dengue network's strongest nodes are PL120, PL25, PL128, PL121, HLL130, PL31, and HLL64. These hubs are deeply rooted in the dengue epidemic's underlying network.

3. Centralization approaches considered for the dengue network

The weighted Newman approach is utilized to calculate closeness centrality, which is displayed in **Figure 10** [15, 22, 23]. Eq. (2) is applied in the closeness centrality. As $\propto$ is set to 0, the shortest path measure is used to determine a node's centrality. Alternatively, if $\propto$=1, the distance is determined using the link weights. Weighted Newman method results show that GL18, GL1, and GL5 are the three closest values. In other words, these hubs serve as the most direct routes for the dengue virus to spread. Based on the number of reported dengue cases, GL18 is the most important node in the network, whereas GL1, GL5, and GL39 are the most connected. It's proof that there are a lot of dengue virus cases in those areas.

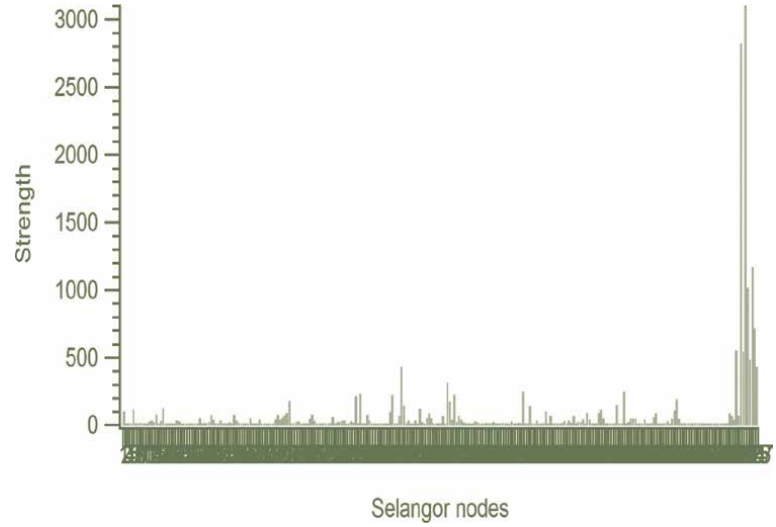

Figure 9.
The weighted Newman method's analysis of the Selangor network's node strengths.

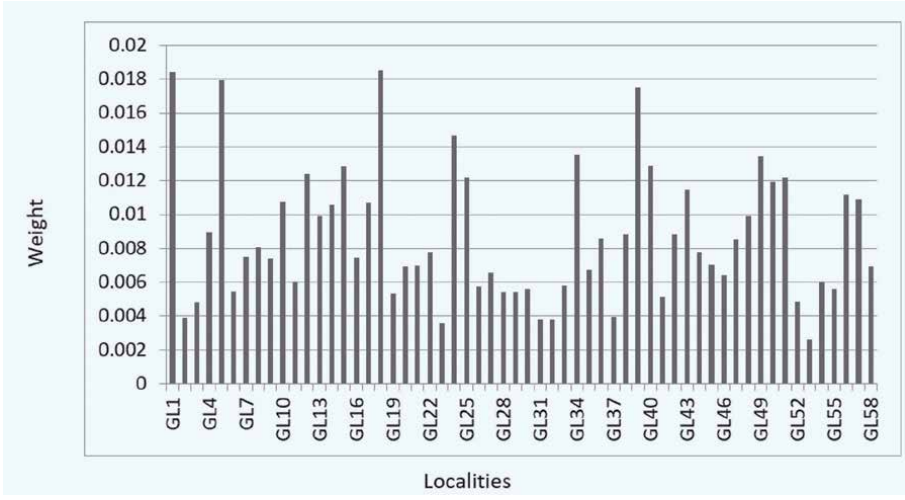

Figure 10.
The Gombak network's weighted Newman centrality in terms of closeness.

Researchers [17, 24–26] calculated a weighted closeness centrality, provided in Eq. (2).

$$C_C^{W\propto}(\mathrm{i}) = \left[\sum_j^N d^{w\propto}(i,j)\right] \tag{2}$$

$C_C^{W\propto}(\mathrm{i})$ is the weighted closeness centrality of node i, and $\propto$ is the tuning parameter. However, $d^{w\propto}(i,j)$ is the weighted distance between nodes i and j.

Figure 10 displays the closeness centrality of just the Gombak nodes, while **Figure 11** depicts the closeness centrality of the entire Selangor dengue network.

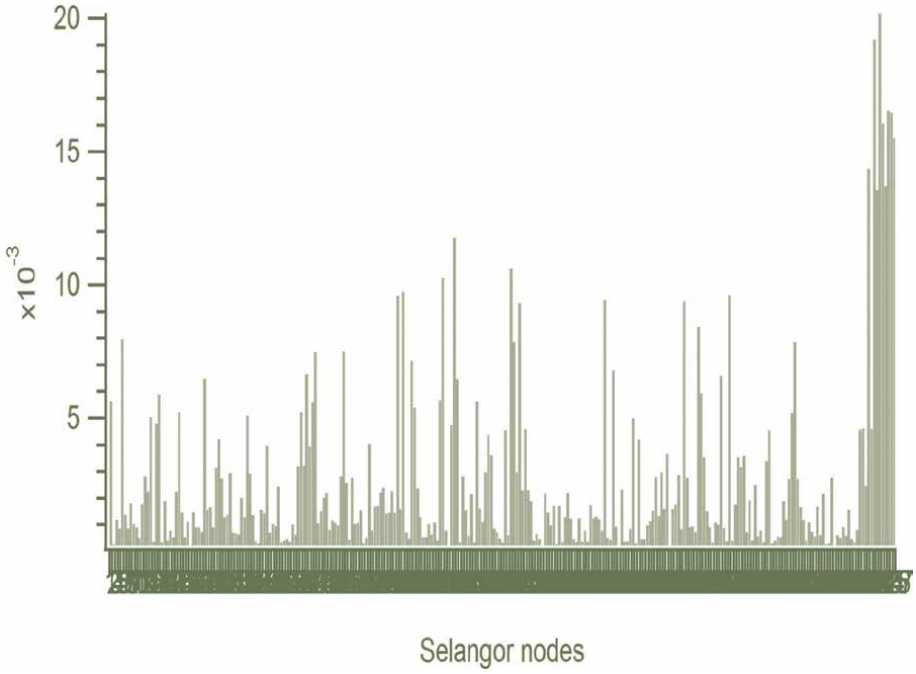

Figure 11.
The weighted Newman method of determining the closeness centrality of the Selangor network.

The x-axis depicts all of the Selangor locations where dengue fever has been confirmed. The closeness centrality measure has been calculated using the weighted Newman projection technique. The nodes with the highest closeness centrality scores in the entire Selangor dengue network are located at PL126, PL31, PL134, PL137, PL127, PL200, SL5, PL28, PL54, and HLL64. This demonstrates that the dengue virus is more likely to propagate from these specific nodes to the rest of the network. As a result, reducing the size of the dengue outbreak requires a concentrated effort at these hubs.

While using the Newman approach, the three closest values are GL18, GL1, and GL5, whereas when using the Sum technique, the top three closest values are GL5, GL18, and GL1. The sum approach of calculating the shortest path has given more weight than the weighted Newman method.

The entire dengue network in Selangor is depicted in **Figure 12** as a betweenness centrality. Weighted Newman analysis was used, with the x-axis representing all dengue-infected nodes in Selangor. The nodes PL126, PL31, and PL137 here have the highest betweenness centrality in the entire Selangor dengue network. This suggests that these three nodes have been crucial in boosting the spread of the epidemic virus across the network.

The generic version of weighted betweenness is Eq. (3) [17–19]:

$$C_B^{w\propto} = \sum_{j}^{N} \sum_{k}^{N} \frac{g_{jk}^{w\propto}(i)}{g_{jk}^{w\propto}} \; \mathrm{j} \neq k \tag{3}$$

$g_{jk}^{w\propto}(i)$ depicts the total number of the weighted shortest paths between two nodes, while $g_{jk}^{w\propto}(i)$ represents the number of those paths that pass by node i.

Figure 12.
The weighted Newman betweenness centrality measure applied to the Selangor network.

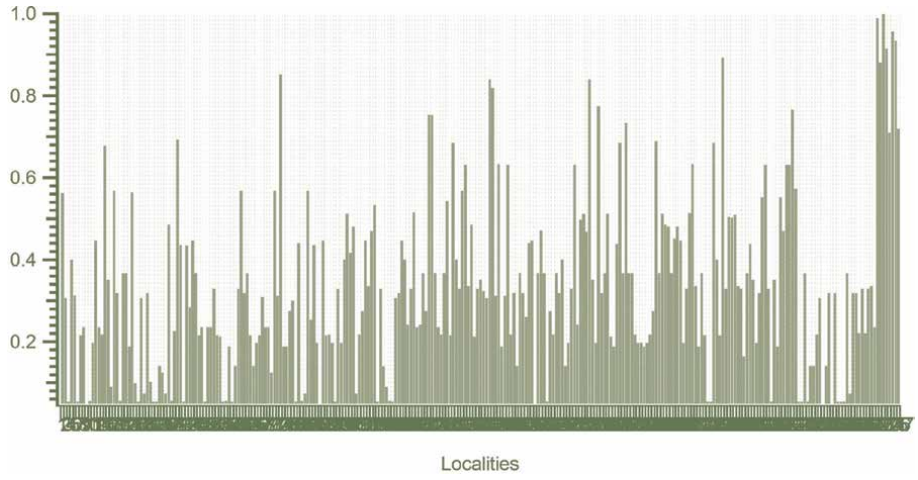

Figure 13.
Eigenvector centrality measure.

In **Figure 13**, we see the outcome of applying the weighted Newman projection method to the concept of eigenvector centrality. The centrality score was computed using Eq. (4). The eigenvector centrality (EVC) measure determines the most significant nodes in a network. Moreover, EVC explains how not every link is of the same value. Based on this centrality study, the most significant Selangor dengue network nodes are 272, 270, 275, 276, 273, 219, 271, 73, 142, 175, 143, 178, 242, and 122. These hubs are significant (in terms of high dengue cases); hence, it is recommended that they be taken into account while designing more effective treatments to curb the current dengue epidemic in Selangor. Eq. (4) defines EVC [17, 19, 24, 25].

$$x_i = \frac{1}{\lambda}\sum_{j=1}^{n} A_{ij}x_j \tag{4}$$

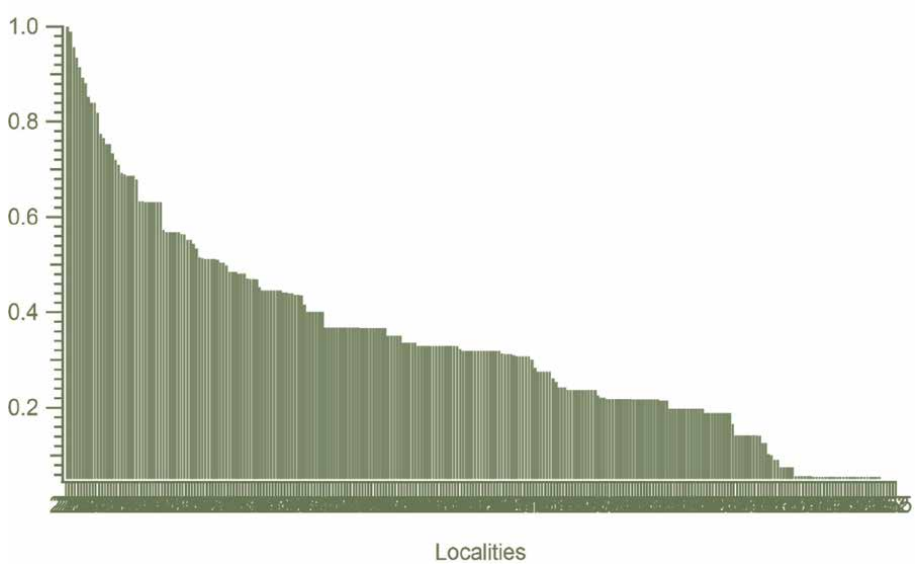

Figure 14.
EVC resembled a power-law form.

Where x_i is the eigenvector centrality of i network, λ is a constant and A_{ij} is the adjacency matrix (A_{ij} = 1 if links i and k exist, and 0 otherwise).

This can be shown in **Figure 14** for the network as a whole using eigenvector centrality, where the power-law form can be observed to indicate the small number of nodes with disproportionately high weights (importance) in comparison to the vast majority of nodes with lower weights [27, 28]. Because more dengue cases appeared at these higher weighted nodes, they should be considered while treating the dengue epidemic network.

3.1 The network density

If we define a potential link as a connection that could exist between two nodes, then the density of a network is the fraction of these potential links that are actually linked. With a density of 0.52 nodes per node, the network is dense. It's clear from this that most of the network's dengue cases are concentrated in a small subset of nodes. **Figure 15** demonstrates the dense nature of the network. For the Gombak dengue network, the y-axis indicates the likelihood of link weights, while the x-axis displays the link weights themselves.

The density of the nodes in the Gombak dengue network is shown graphically in **Figure 16** as a scatter plot, where the x-axis displays the link weight, and the y-axis shows the total number of linkages. As can be seen, the weight of many nodes is low, while the weight of a select few is significant. If this network is partitioned into distinct clusters, as shown in **Figure 17**, then the clusters will be dense. Therefore, treating these clusters effectively is important to disrupt the global dengue transmission system. The y-axis depicts the connection weight in the Selangor dengue network, while the x-axis displays the total number of linkages.

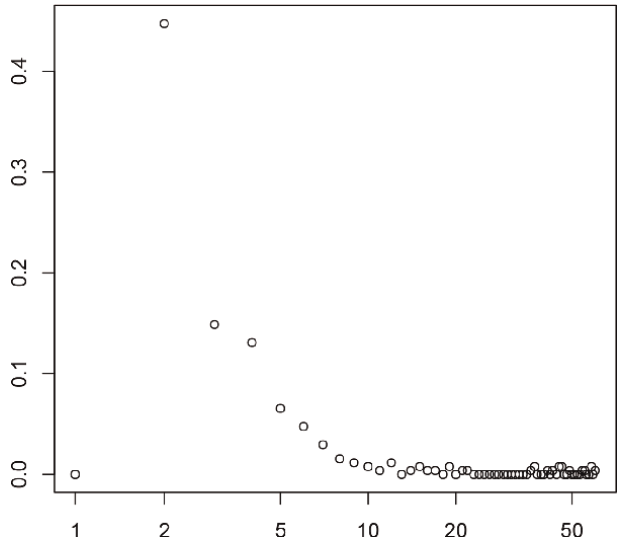

Figure 15.
The network density.

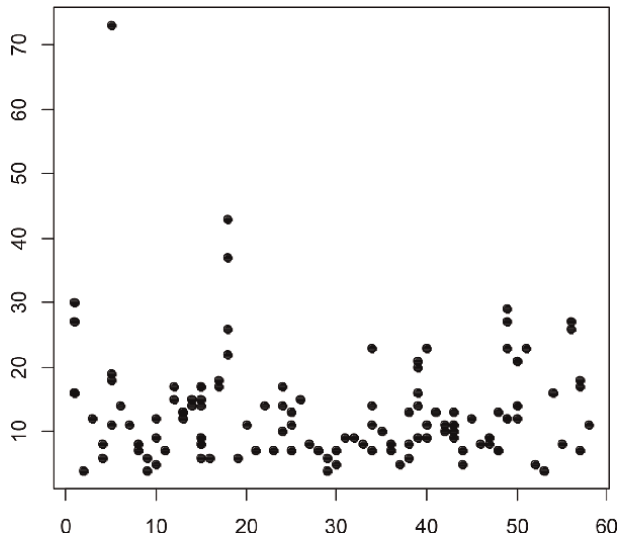

Figure 16.
The density of nodes.

4. Dengue network: scale-free aspects

A few instances of scale-free networks (SFNs) with varying values for the power-law exponent are listed in **Table 1**. Observations of the topological structure of complex systems in several areas of biology have been the focus of many recent studies. When Barabasi modeled the World Wide Web and its hypertext links, he introduced the concept of a spectral family network (SFN) with power-law exponents $\gamma_{in} = 2.1$ and γ_{out}t $= 2.7$. Here, γ_{in} and γ_{out} represent the in-degree and out-degree of the

Network examples	Power-law exponent (γ)
Food web	1.1
Co-occurrence	1.8
Cellular	2.2
Movie actor	2.3
www	2.1

Table 1.
γ for certain representative network systems.

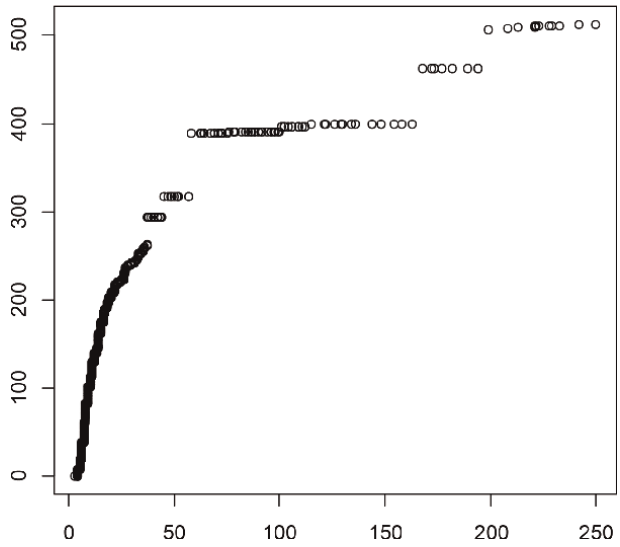

Figure 17.
The density of clusters.

network, respectively [28, 29]. Scale-free power-law distributions were discovered for this social phenomenon, with $\gamma_f = 3.4$ for females and $\gamma_m = 3.3$ for males. Newman modeled scientists as nodes and their scholarly articles as edges in a defined two-mode scientific collaboration network [24, 25]. In this network, the major ties between the two scientists are articles they have co-authored. In the instance of the high-energy physics database, he discovered that the degree distribution of this network follows a power law with the exponent γ = 1.

In **Figure 18**, we see a log–log scale depicting the probability distribution of node strength (the number of dengue cases in various areas of Selangor). If the exponent is roughly near the lower bound of the power-law exponent limit, as shown by the broken line in **Figure 18**, then the network has spatially arranged itself into a scale-free network [15]. As the distribution has a negative slope, the power-law exponent takes on the value −1.9. This probability distribution shows power-law organization across time. Important for determining SFN is the presence of a power-law distribution [15, 24, 27–29].

Moreover, a small number of links carry disproportionately heavy weight relative to the rest. SFN is crucial to addressing the problem of epidemic diseases. If the

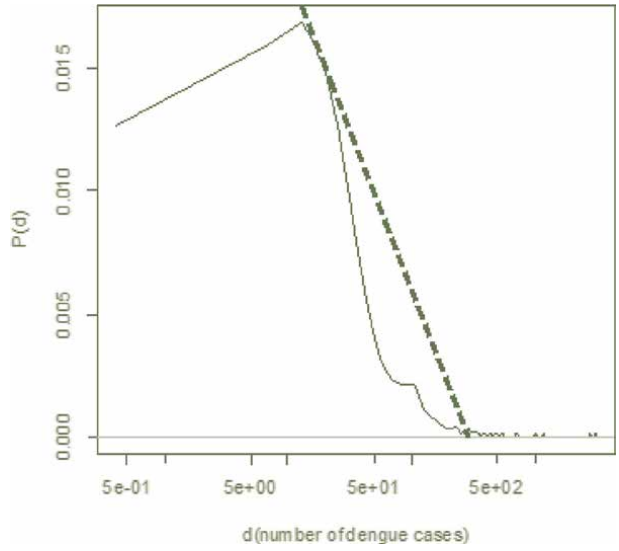

Figure 18.
Dengue fever incidence follows a power law distribution $\gamma = -1.8$.

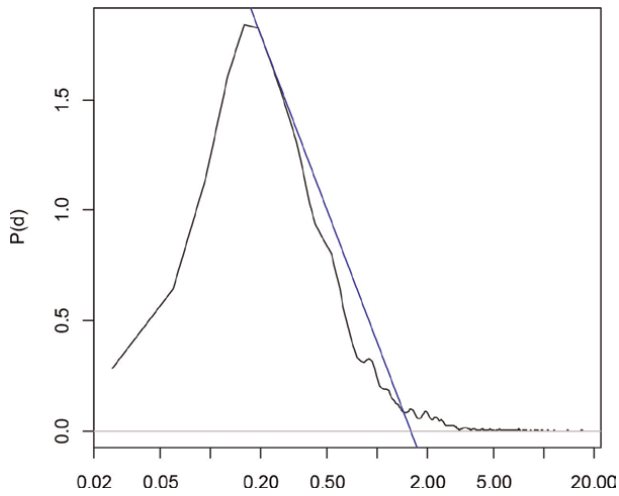

Figure 19.
Node strength by using Newman projection.

epidemic diseases are SFN, this network topology will be more effective in stopping them. In contrast to the random network, targeted attacks can take out central nodes.

The distribution of node strengths reveals the general behavior of the network's strength, which is the weighted degree of dengue cases at each node.

The node strength of the weighted Newman projection is depicted in **Figure 19**; the x-axis depicts the strength of linkages, while the y-axis shows the probability distribution of link strengths. The network's geographical organization resembles a power-law distribution, as represented by the line on a declining curve.

Figure 20 shows connection strength and probability distribution on the x- and y-axes, respectively. It's important to note that the logarithmic scale is used here.

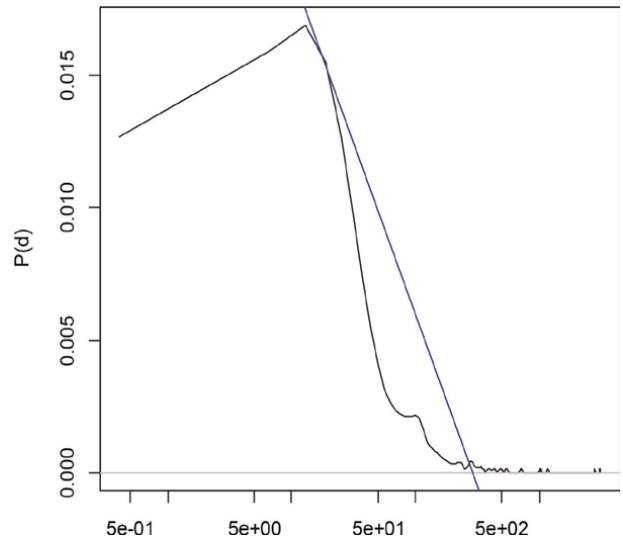

Figure 20.
Node strength of Sum projection.

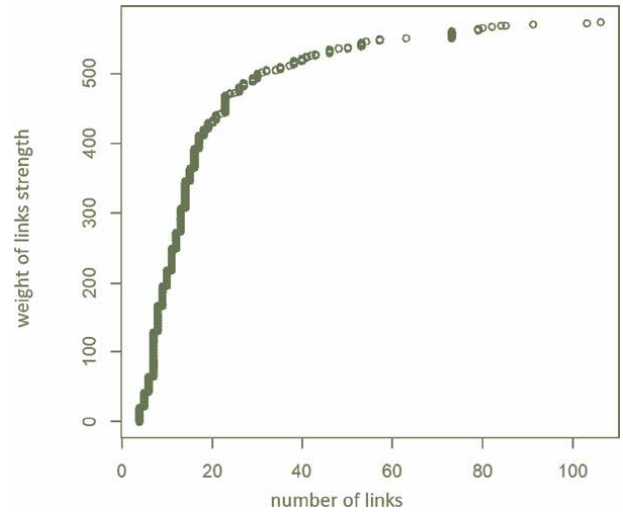

Figure 21.
Weight of links.

A power-law distribution can be seen in the network's geographic organization, as represented by the trendline on the decreasing curve. As this graph demonstrates, however, the dengue virus particularly hits hard only a small subset of nodes. Heavy tails and right skews were seen in the link weight probability distribution.

In **Figure 21**, we see a representation of link strength as a linear function of link weight, where the x-axis indicates the total number of links, and the y-axis reflects the strength of individual ties. It shows that a small number of links in this two-way network have disproportionately impacted the entire network. There are only a handful of major hubs where dengue fever has been widely reported.

4.1 Vulnerability of nodes shown on actual map

Fifty-eight affected nodes are detected in Gombak, Selangor. The dengue-affected locations are shown on the Google Map, highlighting the Gombak area. In **Figure 22**, the Gombak boundary is shown with a curvy red line spotted with a red arrow, and the dengue-affected areas of Gombak are shown in small circles labeled GL1 (Gombak Locality 1) until GL58. Dengue hotspots are displayed here as distinct groupings on the map. GL15 and GL18 are the primary nodes in this cluster regarding the total number of dengue cases reported. Other clusters include GL19, GL20, GL22, GL29, GL6, GL15, GL16, and GL17 [23]. The nodes GL23, GL39, GL40, GL45, GL01, GL38GL53, and GL52 form a second cluster, with GL01 and GL39 serving as its primary hubs. GL05 is the central core of this cluster. However, other clusters such as GL49, GL50, GL 7, GL5, GL10, and GL54 are also visible.

Using a red circle, we have drawn attention to the five major centers (in terms of high dengue-affected cases) in **Figure 23**. These five nodes account for 8% of Gombak's total dengue network. The nodes GL01, GL5, GL15, GL18, and GL39 are colored red. Taking the total number of dengue cases into account, it becomes clear that these are the epicenters that must be addressed to control the spread of the disease. A higher-than-usual number of dengue cases indicate that there are more *A. aegypti* in the area. In addition, these nodes play a crucial role in dismantling clusters, allowing for the elimination of particularly large clusters from the network as a whole.

If this 8% of the network's nodes, symbolized by the green circles in **Figure 24**, are fixed or removed from the network in the future, then this destroys 34% of the dengue network. This is a good illustration of the effectiveness of a targeted attack that is more useful in a scale-free network than a random one.

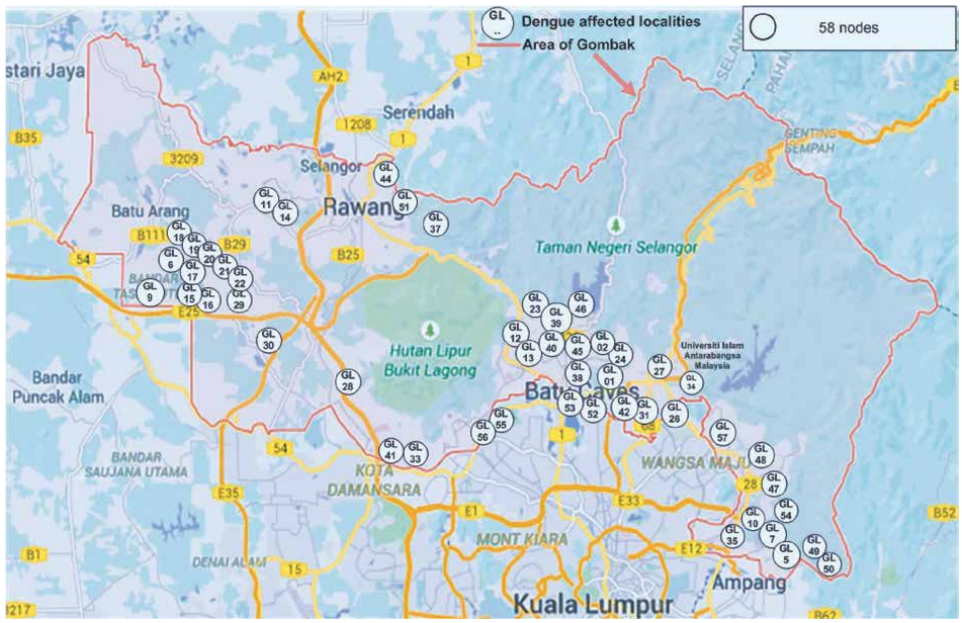

Figure 22.
Gombak dengue map.

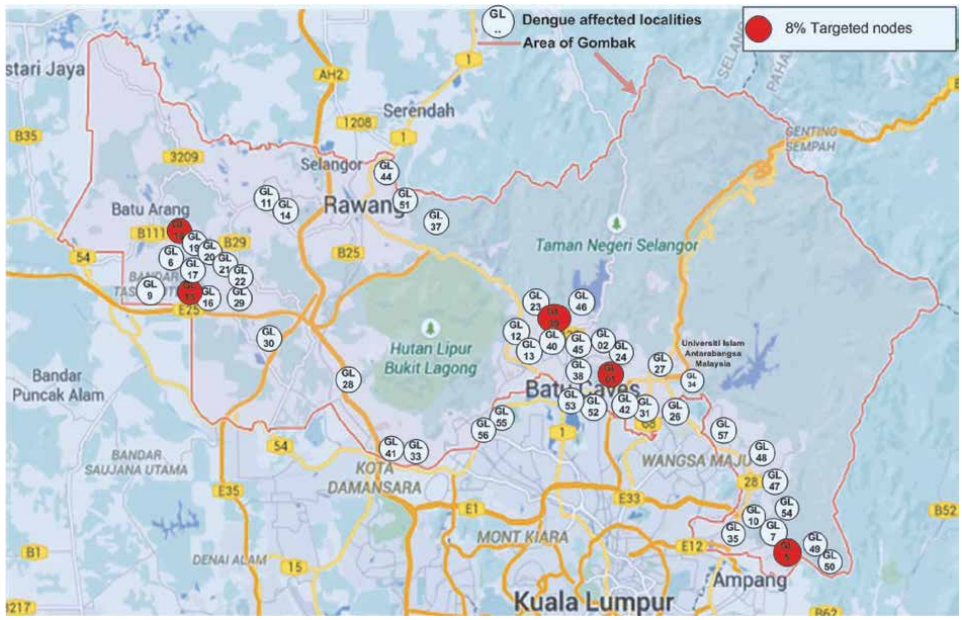

Figure 23.
Targeted 8% nodes (red-colored localities).

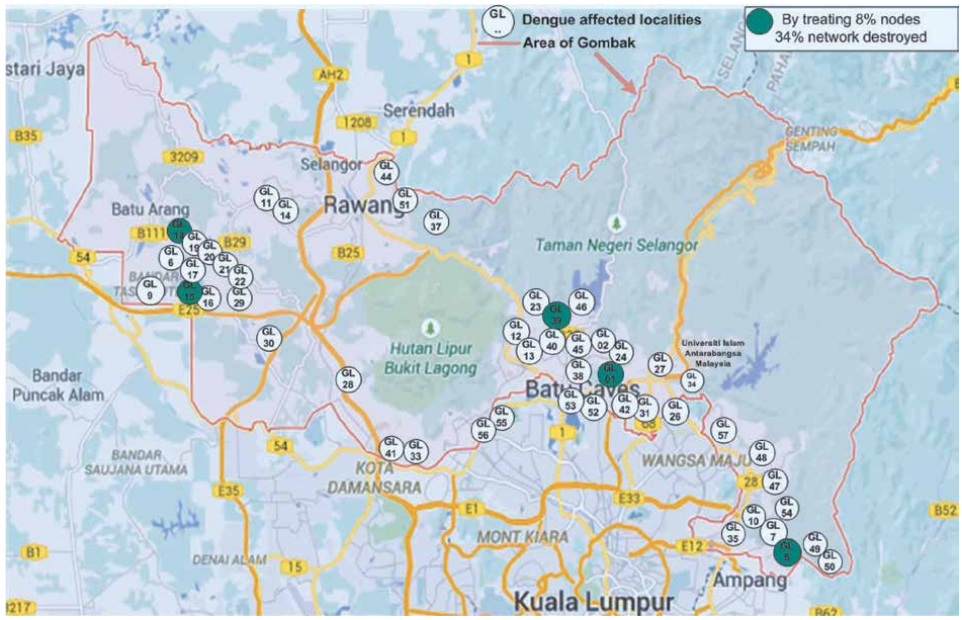

Figure 24.
Targeted 8% of nodes are recovered/removed/treated.

After the targeted elimination of 8% of nodes in the dengue epidemic network from **Figure 24**, the remaining 66% of network is shown on the real map in **Figure 25**. Other nodes in the network that serve as focal hubs have also been identified. This strategy can slow or prevent the spread of the dengue virus. It has been analyzed that a

Figure 25.
Remaining 66% of network.

5% targeted approach created equivalent consequences to 65% of random attacks on the network. Accordingly, rather than dismissing this network as random, it should be treated as a targeted attack that is more powerful in the scale-free network.

5. Conclusion

Dengue fever poses a significant global burden and new challenge to health policymakers worldwide. Despite the many attempts made to combat *Aedes aegypti* and its detrimental impacts on humans, no definitive victories have been achieved yet, as mentioned in the literature review. We have used empirical methods to describe and evaluate the dengue outbreak as a complex network. The dengue epidemic is established to be a scale-free network using network analysis metrics and robustness under the targeted attacks. The results demonstrated that a dengue epidemic network is vulnerable if they adhere to a scale-free network structure.

Furthermore, the study results indicated that eliminating a small percentage of focal hubs destroyed a big part of the network, demonstrating a feature of scale-free networks. The findings revealed that 8% of network nodes, that is, GL01, GL5, GL15, GL18, and GL39, were removed from the Gombak network, resulting in the destruction of 34% of the total network. Dengue network modeling and proof as a scale-free network will contribute to the body of knowledge on complex networks.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 12

Mapping the Dengue Cases Distribution with Google Earth Pro™, Geocoding Attributes Tables

Juan Gabriel Ledezma Acevedo

Abstract

When the epidemiologists need to analyze the distribution of cases in a study or the outbreak trend of cases over time, usually they use graphics for representing the magnitude over time (by incidence and prevalence), tables for describing the variables of the affected people (by race, age, sex, weight, and social condition), and maps describing the spread of places and distribution over time. The technological advances gives most people access to latitude and longitude in smartphones and easy access to a GIS-like free software such as Google Earth™ (GE), an intuitive and effective program for a fast map of the case addresses geocoded, an easy way to display layers imported from formats like Shapefiles extension, and showing over those layers Excel tables with the patient variables and geocoded data from the sheet. Besides, it gives the availability of saving the spatial data with the variables, in files that can be mailed and displayed in smartphones and PCs with Google Earth installed and with outcomes that have a format compatible to GIS classic software.

Keywords: epidemiology, spatial analysis, mapping, geocoded attribute tables, arbovirus, dengue, google earth pro™, geographic information system

1. Introduction

Geographic information systems (GISs) are commonly used by professionals for analysis of geocoded data over maps. But these software are for specialized and trained personnel. Some of them like Arc Gis™ are licensed and have an important economic cost; some other like Quantum Gis™ are open source but not easy to use without appropriate training; some other like EpiInfo™ allows to create variable income of cases and associate them with the geocoded data.

But Google Earth Pro™ has proven to have an extra advantage over classic GIS software. It has the possibility for searching places by typing the name while using it for mapping and also by copying the geocoded data from the search bar placemark, which appears on the surface of earth satellite imagery or the commercial sites in search. So, it is very easy to locate reference places like parks, hospitals, or buildings and approach the address of a patient's home, and this way, the epidemiologist can make the mapping free and easy geocoding of the attribute of the table linked to the corresponding marks in the map.

In 2013, the author of this chapter worked as an epidemic surveillance professional for the government health ministry and tried to give a better representation of the affected places in a rural town, Nandayure, located in the Guanacaste region of Costa Rica, Central America. That region is one of the 3 regions from Costa Rica that usually concentrates over 80% of dengue cases among 9 regions of the public health system [1] and is endemic for dengue fever and other arthropod-borne viruses like chikungunya and Zika, but not for malaria or yellow fever.

So the author of this chapter reported the findings to the director of the office and with another office partner created an original paper of the new case of use of GE™ for mapping cases of dengue, with the combination of excel table of attributes, just like joint function in GIS classic programs but easier.

The occurrence of outbreaks motivates epidemic interventions, in order to control the spread of the viruses and to avoid more neighborhoods and other towns from getting affected. So, the map of the incidence every day or week permitted to make an analysis of the magnitude of the spread for targeting a more extensive application of insecticides [2], and this way it helped to have a lower cost and more effective control of the adult mosquitoes, near the affected person.

The incidence of arbovirus-infected patients in a medical center implicates a report ticket submission to the surveillance in 24 hours after medical assistance in Costa Rica's health system, because this establishes sanitary legislation and regulations [3, 4], and 48 hours after ticket reception the vector control health workers must start investigation, looking for breeding sites of *Aedes sp* and applying insecticide in the house and surrounding houses. Mapping cases of dengue fever is important and necessary for distribution surveillance. But is not acceptable by croquis, or drawings that lack of adequate scale. Especially when everyone has access to satellite imagery free and easy with GE™.

The use of GE™ for the mapping of cases became a case study, and the advantages found in the software were important for improving the quality of representing outbreak advance and control. Because of the images that were offered by the software, it made easier it to save and compare the data over time. Intuitive interface made easy to mark a place with a click of the mouse, and right click permitted us to see the altitude, latitude, and longitude of that mark; rename, cut, and copy; change position; add an image; and so on, just like moving a file in the desktop screen of a PC. The case study becomes a case of use, when the software is commonly used for technical reports, with maps showing the satellite image quality, with title, legend, cardinal points, and scale, by only clicking on a tool bar at the bottom.

At the end of 3 years of use, the author realized that is it possible to take a mark or a group of placemarks from the layer panel, at the left side of GE screen, and convert them it into an Excel sheet, and it is possible to do the opposite: importing an excel table with typed latitudes and longitudes, having as a result a geocoded mapping of dengue patients, showing the coordinates as marks on the satellite images map of Google software. The union of these data from the Excel of attributes with the marks coordinates obtained from health care givers about infected people has an improved database in three dimensions, because it includes time space and person variables at the same time.

All data and variables like the onset of symptoms, address geocoded, and the characteristics of the persons like age, sex, name, date of birth, ID number are provided in a file .text extension that allows to import the data and show them on Google Earth. The label is chosen from the attributes table with the import procedure and with an intuitive navigation as simple as a left click and shows all the data from the patients'

information, and the imported sheet can be saved as a file easy to send as an email attachment and has been proven to be compatible with classic GIS software since 2008 [5].

And that is useful for adding a higher quality map, with marks representing data important for analyzing trends, showing the affected area on a global scale where it is possible to zoom in and out to get a satellite quality view of the streets, relief, and flora. And with great accuracy, it is very easy to get to the roof of a patient's house just by scrolling with the mouse.

A better way for reporting the epidemic distribution of patients' incidence, with layers that can be displayed sequential weekly or monthly, is by just saving different files according to the needs; this way the epidemiologist can explain the location where the outbreak started and how it increases and spreads, to focus efficiently on the intervention and the resources for the decision makers.

Mapping with GE™ began as a case of use for displaying marks representing sickness distribution in a satellite picture from a visor named GE and became a GIS-like layer viewer, with information about patients who had dengue fever, geocoding the houses where they live for a better epidemic surveillance, mapping and analyzing data according to the neighborhood population, altitude, temperature, and urban population density. That case of use of a free software became an original article [6].

2. Using Google Earth Pro™ (GEPro) as a geographic information system

A spot map is used to display the location of each health-related state or event that occurs in a defined place and time. With rare diseases or outbreaks, each point on the map represents a case. An area map may also be used, which indicates the number or rate of a health-related state or event by place, using different colors or shadings to represent the various levels of the disease, event, or behavior [7].

2.1 GIS + epidemiology

The spatial analysis of epidemic phenomena like uniting space, time, and person with all important variables is the backbone for public health.

The time of the year an outbreak starts is important, including when the increase of the incidence is already known previously, like seasonal flu. And weather-related conditions such as temperature, tropical rainy season, and altitude have influence over vector-transmitted diseases; dengue fever is a seasonal and environment-related incidence sickness [8].

These geographic terms are increasingly finding their way into the epidemiologic literature, as advances in the GIS technology make it ever easier to connect spatially referenced physical and social phenomena to population patterns of health, disease, and well-being. Modern geography allows us to understand the space and how its singular environment has an influence over countries, regions, and places [9]. Matching the spatial distribution of cases and outbreaks to the individual, demographic, social and geographic particularities [10].

The variables about a person and its condition are important; for example, taking care of a pregnant woman in a Zika outbreak is fundamental for avoiding congenital abnormalities; some strategies include giving nets to pregnant women [11, 12].

The unstoppable actualization of geographic information software and the free access to satellite imagery make it easier to epidemiologically apply the advantages.

Besides the description or illustration of one condition, the selection of more affected areas must be the focus for the prevention plans and interventions and for the search of possible solutions to the etiology of the condition [13].

The burden of the disease, the global impact, and the vector-transmitted arbovirus become medullar for the necessary increase of the use for satellite imagery. And the advantages offered by GIS have been promoted by the World Health Organization [14] for the prevention of and attention to dengue.

Pan-American health organizations have promoted the use of GIS as a part of their projects to strengthen the capacities of the governments from North, Central, and South America [15].

Nevertheless, it is uncommon to use a GIS for the "place"-related analysis, and usually, rates of people over place are displayed like the entire geographic area in a code of colors that represents level of affection according to those rates. While some health systems in poor developing countries still have whiteboard croquis, some countries have control over the information of patients for preventing epidemics very strictly, by the use of artificial intelligence like by China during the Covid-19 pandemic. One of the experiences of the use of Google Earth for epidemic surveillance was the controlled isolation of Covid-19 infected people; many countries established quarantines and isolations, and some of them developed software for the geocoded control of the isolated people for the respect of isolations and quarantines [16].

Improving urban Aedes control and achieving a measurable impact on dengue virus transmission require a reformulation of current strategies and a stronger focus on the adult mosquitoes that actually transmit the disease, both lowering vector abundance and preventing human–vector contact [17].

In Costa Rica, surveillance of dengue includes the intradomicile and extradomicile insecticide application for the house of a person diagnosed as likely dengue or confirmed dengue, and sometimes the place of work, schools, and public institutions are also treated. There is control o larval, adult, and breeding sites.

Where a case of dengue reported stays the most duration of time, some surrounding houses are also treated, for the protection against the possible infected mosquitoes. If the person has been in medical assistance, the surveillance structure of the mandatory public health system laws and guidelines include the case report and investigation about the places visited 15 days back. And in the case of an outbreak, mapping the cases through the weeks is mandatory until the outbreak is under control [18]. But mapping is not usually as good as the high level of quality of geocoded satellite imagery as Google Earth offers.

Dengue fever is a vector-transmitted disease. Dengue transmission occurs through an insect vector, predominantly *Aedes aegytpi* but also *Aedes albopictus*. Environmental parameters, especially temperature and precipitation, affect the demography and behavior of these vectors, making dengue an obvious candidate to investigate the impact of climate on the disease [19].

In 2008, a group of epidemiologists concluded in a *Bulletin of the World Health Organization* about the importance of taking advantage of Google Earth uses and explained how advanced and costly concepts for disease surveillance could turn into an opportunity to apply low-cost tools and solutions. GE™ proved to be an excellent way to develop great alternatives for improving public health by the urban visualized spatial patterns of vector-borne diseases, creating maps showing the location of blocks with dengue cases reported in 2006 for Chetumal and Merida, México. They showed how to draw blocks made with polygons that would be colored for demonstrating where the cases of dengue affected that block. And they added marks

for labeling infrastructure. But they described limited access then, because of limited internet access in developing countries and rural images that were of poor quality at that time. But this limitation with regard to rural imagery quality changed in 2015 after GEPro™ became free to access [20].

Now instead, the case of use becomes an advantageous technique for any user who requires the map to show the distribution of any Excel sheet on satellite imagery quality. The next is an explanation about the step-by-step process for mapping the entire Excel with attributes and the geocoded address, based on the experience of the place distribution analysis obtained after mapping for each patient reported as a case of dengue fever to the surveillance system, in a rural place from Costa Rica.

To obtain a detailed mapping with all the cases represented over the satellite image, keep all the variables originally in the sheet of the workbook. And for any Excel with geocoded data, patients' workbook can be imported from Google Earth. A sheet with geocoded data of hospitals, or neighborhoods, or houses covered by the medical or insurance service shows the details of each variable of interest.

2.2 Is Google Earth™ an SIG?

2.2.1 Software description

The program starts with an interactive globe, and with the scrolling of the mouse, it can easily zoom in to any point on the surface of the earth, with a high level of detail and accuracy. In the program, the view shows relief; can measure routes and distances between two points; generate polygons and area measurements, circle radius, areas, circumference, and diameters; open other layers; and import other formats like shape files. The layer panel, to the left and down of the screen, lets activate roads, places, photos, announcements, 3D buildings, borders, and labels [21]. But the most important difference or advantage over classic GIS programs is the option to search for a place or direction.

GEPro™ is a layer viewer that could not be considered an authentic GIS, because some spatial analysis tools are not available, neither layer editions nor access to attribute tables. But it is becoming the key for the public health map of events [22]. Besides that, it offers high-quality satellite imagery and powerful search tools, for commercial infrastructure, public infrastructure, and places from local territorial division.

But it is not available to filter marks from a group of placemarks, for creating a new layer based on attributes, like filtering on the classic GIS layer. Hence, if a user has a layer in GE™ that shows the addresses of the dengue cases that have occurred in 1 year, it is not possible to select a filter and create a new layer with only 3 months. The way to create that filtered layer would be to filter in the Excel the 1 year table of attributes, copy in a new Excel only the information from the filter, save the Excel in a (Tab delimited) (*.txt). format, and import the new layer from the GE software.

The procedure in this chapter shows the steps of a case of use where an Excel attribute sheet is imported as a text sheet from a workbook with latitude and longitude to GEPro™. The user has access to the variable tables by clicking the placemarks; the marks can show individually the information from the original Excel attribute table when it is left clicked on the placemark.

But if you need to convert a group of marks that you obtained from GPS into an Excel file, it is possible also by another case of use of GEPro™, moving all the points or marks into a folder in the left viewer, saving as a KML file, and then converting KML into an Excel workbook.

With a right click on the left panel of places for creating a new folder, the placemarks can be moved with the left click to the folder or copied the same way a folder is created and files are moved in the screen of windows; cut and paste with a right click for the mark you will move to that folder created in the placemarks panel.

And with the same right click, save the file into a KML or KMZ format, and that file extension can be used for creating the Excel workbook with all the latitude and longitude data, very easily, by accessing the free Geodata converter on the internet [23]. Then, import the KML or KMZ file saved and convert it into an Excel workbook with coordinates.

There are 3 versions of Google Earth™: normal, Pro, and enterprise open source; all of them are free. But the one described here is Google Earth Pro™. The standard version allows comment maps and creates files KML format, placemarks, polygon, lines, routes, and show layers of maps. The Pro was designed for commercial and professional use but became freely available in 2015 and incorporated improvements about import of maps and digital layers in different formats. The Pro version allows up to 2500 marks sharing and improved the resolution for all images and maps in even rural areas (**Figure 1**), thus enabling the user to explore demographic data, print screen, and make movies off connection [20].

Google Earth frame is based on satellite imagery (Landsat, SPOT, Quickbird) and aerial photography; both of these are periodically actualized. Satellite imagery is full of quality and has great accuracy; thus, a high resolution of Google Earth Pro is guaranteed, the interval of error goes from 0.6 to 1.3 meters [24]. By the year 2016, Google Earth image resolution improved even more, due to Landsat 8th imagery [25].

Some authors indicate Google Earth is not a GIS; maybe it is not a classic one like ArcGis™ or QGis open source, but it is compatible with them because the outcome format KML can be opened on classic GIS software. And it has advantages over classic ones, because it is easy to access, reliable, fast, portable, free, and intuitive and offers powerful search.

Figure 1.
A scale map of a rural neighborhood created from Google Earth Pro™. Figure created by the author of this chapter.

3. Procedure concerns for mapping the Excel with geocoded addresses

3.1 Installation

First of all, one must install Google Earth Pro™ by downloading the program from the official website [26].

Once you start using it, it is very important to go to the tools, select options, and change the Show Lat/Long options, choosing decimal degrees, because the program begins when it opens the first time, showing degrees in minutes and seconds; this is a format that is not compatible with most of the cellular coordinates that often are shared by message services like WhatsApp location sharing.

3.2 Excel considerations

The second basic concern is to check the Excel workbook, which must have only one sheet, because the import of a file is only for a singular sheet. When the Excel has the latitude and longitude separated by comma, the file imported in GEPro™ will have a problem for the decimal reading; thus, the decimal must be separated by a point. For an easier and faster change, go to the search option in Excel and choose the replace option; there you have to type a comma for the search and type a point as the replace; it is going to change all the comma-separated decimal to point-separated ones (Video 1 available at: https://youtu.be/29BR7NM52XY).

3.3 Mapping patient's address in Google Earth Pro™

Epidemiologists are daily familiarized to databases that show variables of the victims or affected cases; most of those data can be exported to other formats and there is always an Excel workbook option outcome, which contains the list of patient information like ID, date of birth, symptom onset date, sex, work, address, and more.

But if the direction is from a place the user does not know or is located in another city, or if the epidemiologist mapping that case is new in the town, the map of cases could be a big deal. In this case, the user of GEPro™ can take the address and type in the Google Earth search panel the name of the building where the person reported to be living at the moment of the medical attention or the coordinates sent from a WhatsApp message of the patient's location.

For example, if the address is 600 meters north from the church, it is so easy to place a mark by typing the name of that church in search option, then with the rule measure, the 600 meters in the north direction guided by the cardinal points is shown on the screen. The rule is one of the most important in the tool bar, which helps to place a mark based on the address.

In the approximated measurement, the user sets the placemark and copies the coordinates of the latitude and longitude, so these values must be pasted to the latitude and longitude column in the Excel, with a negative sign in the case of western hemisphere and southern hemisphere. This way, the epidemiologist goes on to add more geocoded addresses to the Excel due to the cases.

3.4 Correcting an address

Sometimes, trying to find the house of the person with dengue fever symptoms, for the necessary breeding site assessment and insecticide intervention, can be

difficult because the direction given by the person to the medical service was not good enough because of missing information, or wrong cardinal point reference, or not being the current address anymore. And the health worker can ask the patient for the coordinates of the current address by cellular phone to relocate the geocoded position of that house. That way the arbovirus assessment can be more accurate, and the change in the address of patients can be done right and easy. Just change the data of the latitude and longitude columns from the excel sheet and this way correct the wrong address and save the workbook for importing again from GE™.

An experience of isolation and control of transmitted disease with geocoded data, occurred when a tourist visited for vacations during pandemic between 2020 and 2021. Before leaving the country it was necessary a negative test for covid, but if the person tested positive to Covid-19, that tourist had to stay more days in the country for isolation, and sometimes needed to find another place to move, because the next days, the room would be reserved for other tourists, so the Covid-positive person sometimes had to change the place of isolation.

In this case, the coordination with the surveillance system may include the patient asking for permission or communicating to the professional epidemiologist of the surveillance system to move to another place. And one of the ways to confirm the new location of isolation could be by sending a mail or message to confirm the new place coordinates to continue isolation; the person can be asked if they agree to send a message with the location from the cellular phone through a WhatsApp message or email. Sending location before getting out of the room and when arriving to the next room or hotel can help the health system give better and faster assessment in the case of an emergency.

3.5 Importing data from Google Earth

The Excel workbook must have only one sheet, and for the import of the data, we have to save that workbook as a copy, in a text format that looks this way: (Tab delimited) (*.txt). That file is the one for import; in Google Earth Pro™, go to the tool bar, File/Import; there the file will be shown while selecting the generic text *.txt *.csv format that corresponds to the name of the workbook tab delimited saved.

When all the addresses are geocoded with corresponding latitude and longitude for each case in the Excel workbook, it is very important to fill all empty or missing data of the sheet with alternative words, like typing null, 9999, empty, missing, or any word that completes that missing data, because empty cells can make the display of variables misplaced, when importing the workbook from Google Earth.

3.6 Creating layers with filtered data from Excel sheet

If epidemiologists, health providers, or any user has all yearly data of dengue cases in an Excel workbook and wants to create a layer with only one-month cases of dengue, to see a one-month layer of marks, one must filter the month in Excel, copy the elements, and paste them on another workbook. And save this new workbook as a layer of cases with the only filtered month as another text sheet or (Tab delimited) (*.txt).

3.7 Opening the layer of marks in Google Earth Pro™ and final steps for the template

When importing, the user must select File/Import/and chose import the (generic text *.txt *.csv) extension; suddenly, a window appears named Import wizard. The user must check or select the delimited tab bottom, and then press next. In the next

window, user must check or select the latitude field correspond to the column with latitude data, and the same for longitude. Frequently, the latitude is the (Y) labeled column and the longitude is the (X) column of data. Then, user must press the finish bottom and create the template style, at this step, it is very important to choose the label selected column, could be names, or maybe the number of the case, in the order they became sick. The user can choose the label to display over each point and can choose the color and the figure for the marks. Having as a result the .kst extension file to save. It appears at the left panel as a GE™ world symbol that contains the marks, to see the marks individually, must double click the GE™ world symbol that contains a folder with all marks. And finally, there is the layer of geocoded addresses, that represent the cases imported from Excel with all attributes (Video 1 available at: https://youtu.be/29BR7NM52XY).

It is very important to know that layers like a .shape extension files can be imported from GEPro™, so the atlas with borders, layers of cities, rivers, and more can be imported and become part of the map with the distribution of cases, represented by placemarks.

3.8 Sending the file by mail and format compatible to GIS programs

Any mark, polygon, route, layer, or group of them can be saved as a file and exported in formats compatible to classic GIS apps and programs; just save the place or mark or group of marks. The format is KML or KMZ file, which can be sent by mail to a person with GE™ or classic GIS preferred program installed on PC, or tablet, or phone. The person will be able to see the saved information display instantaneously.

The public health systems and databases should incorporate that geocoded data for the surveillance of infectious diseases transmission, not only for arboviruses vector-transmitted diseases but also for respiratory and several infections that become of interest, mainly those under international surveillance. For example, the next figure is a print screen of GE™ view, that shows marks with a label, that is the number of the case, and left clicking the mark, displays attributes or information from dengue patient number 40 reported by the medical service from a rural town of Costa Rica, that occurred on 2022 (**Figure 2**).

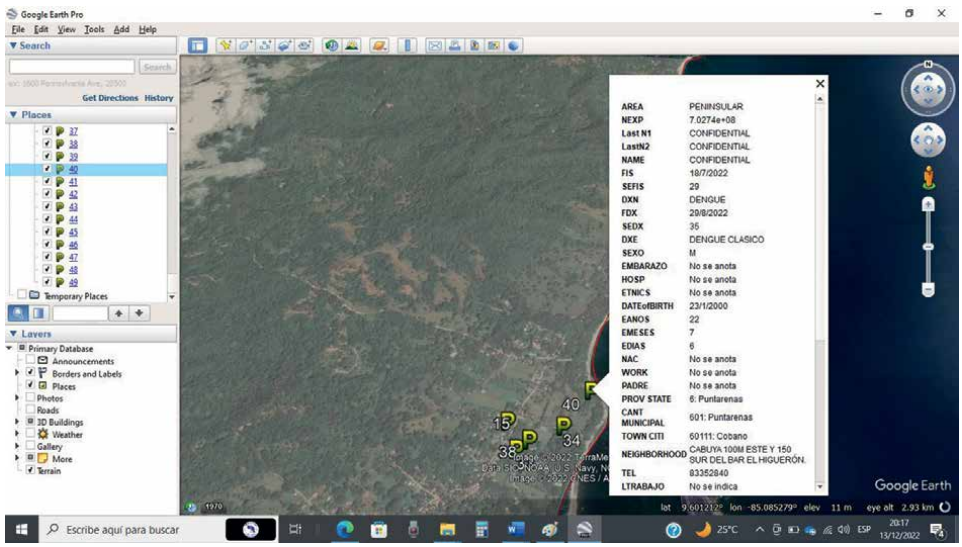

Figure 2.
Print screen of left click over a placemark labeled 40, which represents the 40th case of dengue with attributes shown, numbers arranged by symptom onset. Figure created by the author of the chapter.

4. Conclusions

Any epidemiologist can map the cases of vector-transmitted diseases with an Excel attributes sheet and geocoded addresses, importing the data from Google Earth Pro™, for a better space, time, and person analysis that is free and easy.

Acknowledgements

To the Ministry of health of Costa Rica, where the science, the sanitary legislation, and health regulations have permitted to preserve care of the public health, by the emission of health politics that establish the guidelines for the attention of patients identified by public and private health providers, and for the actions that allow the control of epidemics, and for the opportunities offered by this governmental institution for professional growth, for a great epidemical surveillance system.

Conflict of interest

"The author declares no conflict of interest."

Notes/thanks/other declarations

Thanks to the public education of Costa Rica. Thanks to my family.

Video materials

Video 1. Mapping an excel sheet with geocoded data on Google Earth Pro, for epidemic surveillance. Created by the author for a better interactive user's guide. Available from: https://youtu.be/29BR7NM52XY.

Acronyms and abbreviations

GE™	Google Earth™
GEPro™	Google Earth Pro™
KML	Keyhole markup language
KMZ	Keyhole markup zip
GIS	Geographic Information System
.csv	Comma separated values
.tab	Tabulation separated values
.kst	Google Earth style template
PC	Personal computer
GPS	Global position system
Arbovirus	Arthropod borne virus

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Chapter 13

Genomic Surveillance and Intervention on Dengue Virus in an Urban Setting in the Philippines

Francisco M. Heralde III, Glenda B. Obra and Maria Perlita B. Apelado

Abstract

This is part of the ReMoVE Dengue Program (i.e., research on mosquito, virus, and eco-socioeconomics of dengue) initiated under the auspices of the National Research Council of the Philippines, which started in 2012 aimed to develop locally adapted technologies, products, and systems, which would control the spread of dengue virus and reduce the eco-socioeconomic impact of dengue. Here, will be reported the results of the genomic surveillance of community-collected mosquitoes from a dengue hotspot community of Barangay Old Balara in Quezon City, Philippines using serotype-specific dengue PCR, and the developed antisense RNA product platform for dengue virus control based on surveillance results. Implications and recommendations for this work are outlined.

Keywords: genomic surveillance, dengue PCR, dengue hotspot, antisense RNA, virus control, surveillance-based intervention

1. Introduction

Dengue remains to be a major problem in several Metro Manila cities and in the entire country. Since 2011, dengue cases in the Philippines continue to rise at an average rate of 3900 cases per year, with recorded cases of 34,940 in 2022 [1]. Among the regions, Central Luzon is with 6641 or 13%; Central Visayas, 6361 or 12%; and Zamboanga Peninsula, 4767 or 9% were the top contributors [2]. The increasing cases reflect a number of underlying scenarios and causes, which are difficult to pinpoint, although, one thing is clear, the current strategies for control and mitigation may not be as successful in containing the growing problem of dengue. The Philippines has stood as first in dengue cases globally, like the first recorded dengue epidemic in Southeast Asia that occurred in Manila in 1954, and the highest dengue case contribution ever recorded globally in 2019 of 437,563 cases [3]. It is amazing that despite government efforts and programs, this mosquito-borne disease continues to successfully become endemic and ravage the population [4]. Perhaps, a series of unfortunate events contributed to this unsuccessful mitigation, like the "lack of empowerment among the stakeholders in taking responsibility for dengue prevention" despite the

Philippine government's established National Dengue Prevention and Control Program in 1993 as well as the failed Dengvaxia vaccine program launched in 2016 [3], although other reasons may underlie this scenario. Nevertheless, optimistic perspectives remain as new research shed light on better strategies for control and mitigation [5]. Among these strategies, the dynamics of the virus-vector interaction and the phenomenon-based targeting may hold the key to dengue's long-term prevention and control (**Figure 1**).

Aedes aegypti is the primary vector of the dengue virus, although *Aedes albopictus* has also been identified as a minor vector [6, 7]. Apparently, as more urban communities expand (i.e., which is a common trend among cities with increasing population) to cover semi-urban, semi-rural, and forested areas, the *Aedes* mosquitos have adapted to survive and breed in water pools and deposit in these areas. The Philippine Department of Science and Technology's Ovicidal and Larvicidal (DOST's-OL) trap technology was adopted in 2011 as a widespread strategy for controlling mosquitos [8]. The OL-trap technology involved the use of agents that can kill mosquito eggs and larvae in stagnant freshwater containers that serve as traps. Meanwhile, another kind of trap, the Orbi-traps has been validated as means to monitor mosquitos in different localities [9]. In particular, the Orbi-trap procedure has been utilized in monitoring A. *aegypti* mosquitoes and correlated with dengue cases in Manila [9]. Following a simple mosquito trap design [10] with modifications, adult mosquitos may be caught and

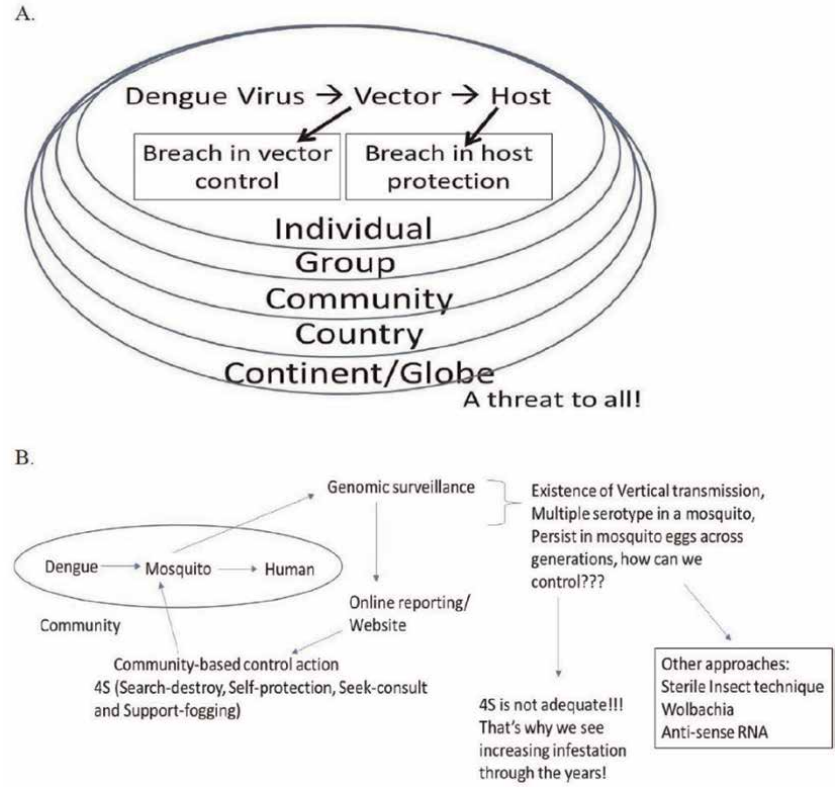


Figure 1.
The conceptual framework for genomic-based surveillance and intervention. A. Dengue as a threat to all with breaches in vector control and host protection. B. Genomic surveillance and intervention as a cornerstone in the fight to control dengue.

morphologically identified. Further propagation of this scheme through a DOST invention [11] could be augmented by monitoring the virus present in the collected mosquito through a PCR analysis, thus may give early advice on the type of dengue virus circulating among the mosquito population in the community.

The PCR platforms for dengue virus detection are widely available in various institutions, the academe among others, and may be utilized for community-based surveillance, especially when appropriate community-academic institution link-up is established, especially now with several molecular laboratories with PCR machines being underutilized as COVID-19 testing declines [12–14]. Furthermore, what is needed would be a system for sample collection, the reagents for the genomic surveillance work, and an online-based reporting system accessible to the community, a model considered and espoused in this project [15]. PCR protocols for routine MDRTB, H1N1 virus, and leptospirosis detection have been established in the Department of Biochemistry and Molecular Biology—University of the Philippines College of Medicine (DBMB-UPCM). The laboratory had an extensive experience with MIRU-VNTR analysis and sequence analysis, including the use of appropriate bioinformatic software. Application of similar strategies to the dengue virus would be convenient.

Genomic-based interventions could be installed in the Orbi-traps, such as a mosquito-feeding device, where anti-dengue interventions can be incorporated into the blood formula. Several studies have demonstrated the positive response of mosquitoes in feeding warm blood [16–19]. The feeding device, however needs to be designed, although "blood-filled condoms" have been reported to work in luring mosquitos [19, 20]. Anti-dengue DNA vaccines have also been reported to elicit an immune response in humans [21]. Meanwhile, mosquitos can be ideal vaccine targets, capitalizing on their endogenous defense system to block the spread of the dengue virus [22].

A set-up where a suitable container, such as a condom, with a chemical-based heating system [20] and incorporated with lactic acid and carbon dioxide would be ideal to attract mosquitos to the feeding device and insure consumption of an anti-dengue vaccine. The DBMB-UPCM has reasonable experience in recombinant DNA work, including the design and production of plasmids for various uses, including sequencing, expression, and DNA vaccines. Some constructs reported in the literature can be tested in the process.

DNA vaccines have been demonstrated in the control of West Nile virus *via* vaccination of American robins—the intermediate host involved in viral amplification that is feeding on *Culex* mosquitos [23]. Meanwhile, the mosquito defenses against the dengue virus have been studied to involve the JAK–STAT pathway, where a specific RNAi-based inhibition of PAIS or protein inhibitor of activated STAT results in increased survival of mosquitos from bacterial or viral challenge [24]. Similarly, an oral administration of DNA nanoparticles synthesized by complexing plasmid DNA with chitosan, a natural biocompatible polysaccharide, was shown to result in transduced gene expression in the intestinal epithelium [25]. Furthermore, the *Wolbachia* wMelPop strain, an endosymbiotic bacterial pathogen was found to be transferrable from *D. melanogaster* to the mosquito *A. albopictus* with the consequential effect of reduced longevity and fecundity, and high embryonic mortality [26]. While in *A. aegypti*, increased locomotor activity and metabolism were reported [27]. Thus, a protocol involving orally delivered DNA construct that would modulate the mosquito immune response combined with bacterial coinfection would manage dengue viral and bacterial residency in the vector, thus presenting an avenue for combined

antiviral and bacteria-based control. This concept was applied in a study, where a cationic liposome was utilized to deliver an expression construct with the gene for *Ae. aegypti* thioester-containing proteins (AeTEPs), (i.e., involved in the control of flavivirus infection), resulting in reduced dengue virus infection [22].

This project was proposed to add value to a program of wide-scale mosquito monitoring by surveillance of the virus present in the collected mosquito by PCR analysis of its DNA/RNA extract and provide advice on which dengue type is circulating in the mosquito population of a given site. Furthermore, in the Orbi-trap, a mosquito-feeding device could be installed, where an anti-dengue DNA vaccine could be introduced.

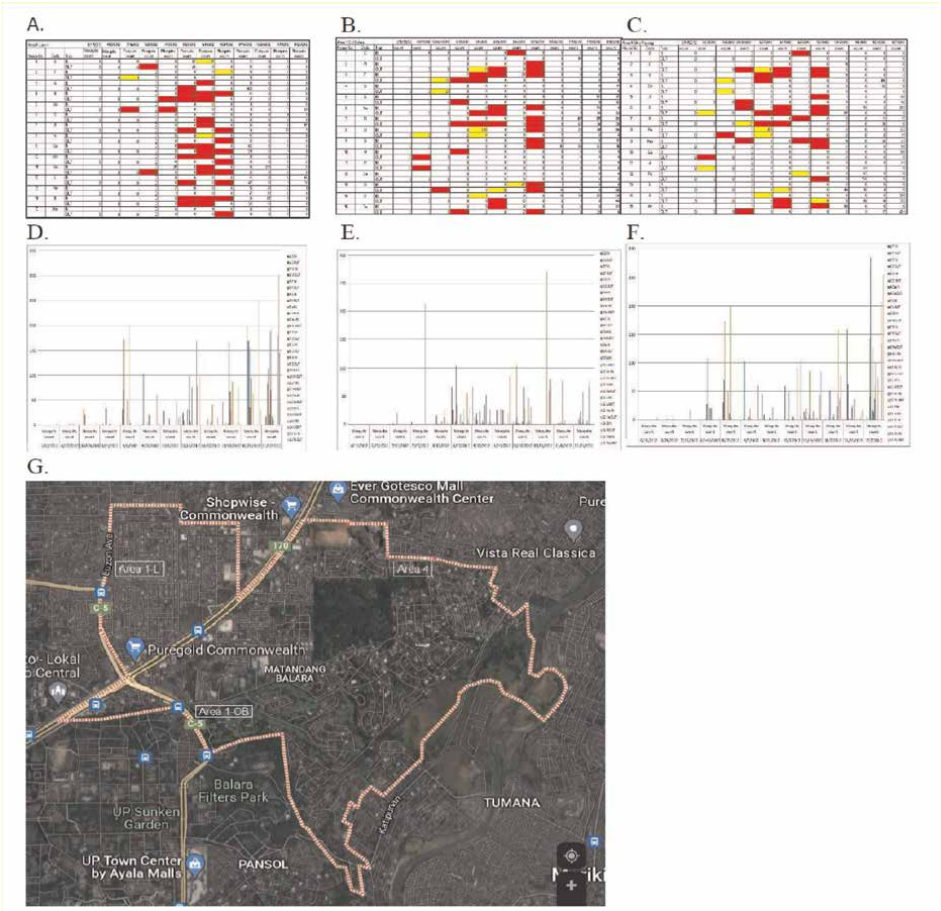


Figure 2.
Mosquito collection summary and dengue PCR test results of the three sampling sites A. Luzon, B. Old Balara, and C. Sitio Payong in year 1. The red highlights indicate the households are positive for dengue, and the yellow is negative for dengue. Entries with zero values and yet show red highlights indicate the late emergence of mosquitoes from the collected sample, which comes from the eggs. Note the increasing number of houses with positive for dengue more than 3–4 houses, starting from the sixth to the eighth collection. There were houses that consistently show dengue positive mosquitos. Mosquito counts of the three sampling sites: D. Luzon, E. Old Balara, and F. Sitio Payong. Most of the houses yielded in their vicinity (outside) a count of 0–50 mosquito individuals (i.e., larvae, pupa, and adult), a moderate number of houses with 50–100 counts, and a few houses with 100–150 counts across the different collection times. In terms of counts greater than 150, Old Balara had more instances across the different collection times followed by Sitio Payong and lastly by Luzon. G. Project experimental site in Quezon City, Philippines [28].

In the first year of the project, a study to evaluate a holistic vector control program was embarked, which involved strategies, such as genomic surveillance and intervention, herbal-based larval destruction, irradiation-induced sterility of mosquito, and biocontrol-organism based larval control among others (ReMoVE Dengue Program—research on mosquito, virus, and eco-socioeconomics of dengue). Mosquito traps were installed in three sites in Barangay Old Balara, Quezon City, at 15 houses per site and 6 traps (i.e., 3 inside and 3 outside) per household, with GPS coordinates determined. The captured mosquitos were counted bi-weekly from May 2012 to January 2013 and serotype-specific dengue PCR was used for monitoring viral presence. In years 2 and 3, monitoring work was continued in the three sentinel sites (i.e., three houses per sentinel site). Also started the development of anti-dengue dsRNA as well as the validation of trans-ovarian dengue transmission and virulence testing in a mouse model. This report outlined the findings of this community-based study.

2. Materials and methods

2.1 Sampling and nucleic acid extraction

The experimental site has been identified and mosquito traps have been set up in a total of 45 houses (15 houses per site with 3 traps indoors and 3 traps outdoors). The sites were Area 1- Luzon, Area 1- Old Balara, and Area 4- Sitio Payong, with prior consultation and approval of the Quezon City Health Department (**Figure 2G**). Two Orbi-traps per site and six OL-traps per household (3 indoors and 3 outdoors) were installed.

The Barangay Health Workers (BHW) together with the project science research assistant conducted the biweekly sample collection. The Orbi-traps were utilized to monitor the adult mosquitos, while the OL-traps were utilized to monitor the egg and larval stages. The collected samples were sent to the DBMB-UPCM, where all field samples were stored, counted, identified, and processed, for RNA extraction and dengue detection by PCR. A small area (i.e., a mosquito insectarium, **Figure 3B**) has been set up for growing larvae collected from the field prior to molecular analysis. Preserved samples per collection receptacle (Orbi-trap or OL-trap at 1–50 mosquitoes and in cases exceeding 50, random sampling was done) were pooled and processed for RNA extraction using Qiagen RNEasy Kit following the manufacturer's protocol.

Reagents, materials, and samples were procured for the project, including an electronic air temperature and wind velocity meter, mosquito traps, primers, and laboratory and office supplies.

2.2 Detection of dengue virus by reverse-transcriptase-PCR

2.2.1 First strand synthesis

Following the protocol of Lanciotti et al. [29], target viral RNA was converted to a DNA copy (cDNA) using reverse transcriptase (RT) and the dengue virus downstream consensus primer (D2). The first strand synthesis was done using the Omniscript or Promega (Qiagen, Macare Philippines, Golden Bat (Far East) Inc., respectively) following the manufacturer's protocol.

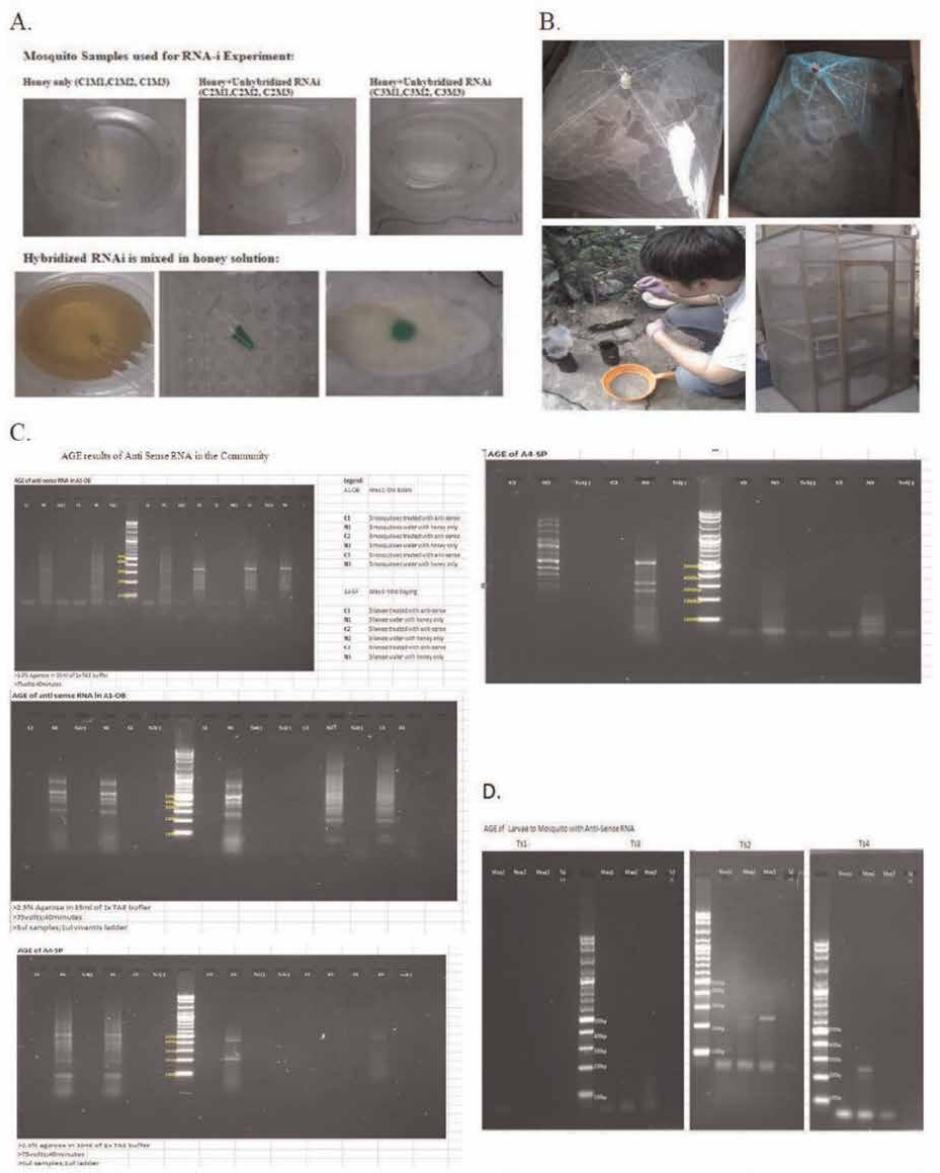


Figure 3.
Antisense RNA trial testing on mosquitoes in the community. A. Preparation of antisense RNA formula with honey solution. B. Simulated mosquito setup in the community for the trial testing, collection of specimens from ovi-traps, and the insectarium used in the lab for handling and hatching the specimens. C. Electropherogram of dengue-PCR results of antisense RNA fed vs. non-fed specimens. Representing TS1-serotype 1, TS2-serotype 2, TS3- serotype 3, and TS4-serotype 4 conducted in different locations, Area 1—Old Balara and Area 4—Sitio Payong. Notable is the specific disintegration of signals in fed vs. non-fed and brown sugar only. D. Dengue-PCR of larva specimens from fed samples with the persistence of serotypes 2 and 4.

2.2.2 Polymerase chain reaction

Serotype-specific amplification was done from the cDNA template with the upstream dengue virus consensus primer (D1) and the downstream serotype-specific primers (i.e., TS1, TS2, TS3, and TS4). Target cDNA was amplified in 10-μl volumes

containing the following components: 10 mM Tris (pH 8.5), 1.5 mM MgCl2, 10 uM each of the four deoxynucleotide triphosphates, 10 pmol each of primers 1 (D2) and 2 (i.e., either of TS1, TS2, TS3, and TS4) and 0.5 U of TopTaq (Qiagen, Macare Philippines) or GoTaq (Promega). The PCR reaction profile consists of the following: initial denaturation (94°C, 1 minute), and then to proceed with 35 cycles of denaturation (94°C, 30 s), primer annealing (55°C, 1 min), primer extension (72°C, 2 min), and followed by a final extension step of 72°C for 10 min.

2.2.3 Agarose gel electrophoresis

The PCR products were analyzed by gel electrophoresis on a 2.5% agarose gel (Vivantis) containing Gel Red (0.5 ug/ml), with the settings of 75 volts, for 40–45 minutes. A band on the agarose gel of the correct size was interpreted as a positive result. A faint band of the correct size was considered an equivocal result.

2.3 Anti-dengue dsRNA study

Primers targeting conserved regions in the UTR-Core gene were designed. The primers are:

UTR36 5′-GCTTAACGTAGT(T/G)CTAACAGTTT-3′ 62 deg

CAP521rc 5′-AACATGTGCACCCTTATAGCGA-3′ 64 deg

T7UTR36 5′-GAAATTAATACGACTCACTATAGGGGCTTAACGTAGTKC-TAACAGTTT-3′

T7CAP521 5′-GAAATTAATACGACTCACTATAGGGTCGCTATAAGGGTG-CACWTGTT-3′

The translation product of the target region is shown in **Figure 4** panel A, and the region targeted in the viral genome is shown in panel B. The primers are used in the subsequent cDNA and dsRNA synthesis of RNA extracted from female *A. aegypti* mosquitos infected with DENV obtained from Barangay Old Balara, Quezon City. The

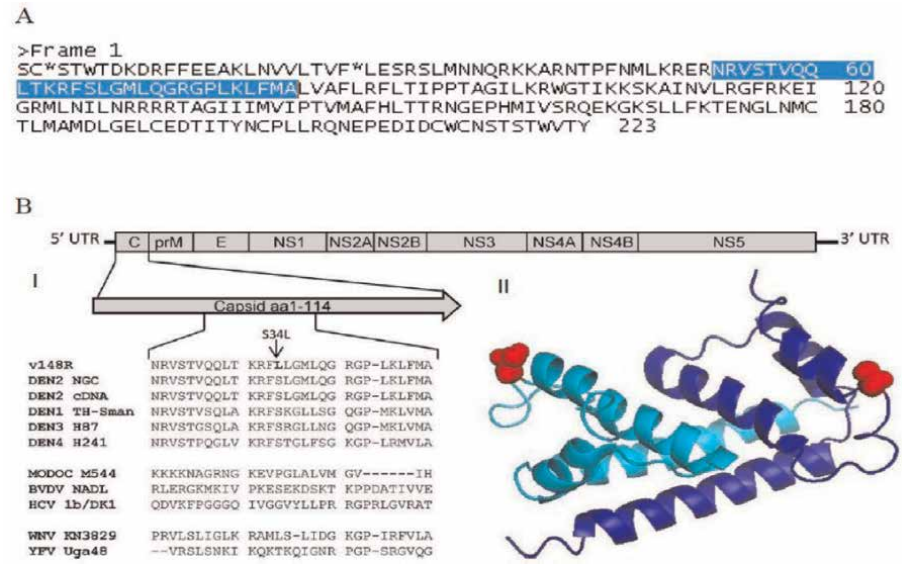


Figure 4.
A region in dengue viral genome targeted for dsRNA.

synthesized dsRNA is administered to a group of mosquitos alongside a parallel treatment of control RNA with mosquitos coming from the same population. The presence of the dengue virus serotypes, after 2 days posttreatment, is detected through nested PCR.

3. Results

3.1 Sampling and nucleic acid extraction

In the initial year of community genomic surveillance, 12 sampling events were conducted. Mosquitos from the first to the twelfth sampling were tested for dengue-PCR using standard protocols as described. An increasing trend of more than 3–4 houses per site was found positive for dengue (Panel A–C, **Figure 2**). Twelve sampling data points for Area 2- Luzon, Area 1- Old Balara, and Area 4- Sitio Payong were uploaded to the project website [15]. The goal was to provide online access to the Barangay Health workers and use the information in their search and destroy program for the breeding ground of the mosquitos. This way, their campaign will be focused on the critical spot in the community. The other objective also was to guide the community as to which areas to avoid as possible exposure sites for the dengue-infected mosquitos.

The mosquito counts from the first to the twelfth collection were plotted as shown in **Figure 2** (Panel D–E). It can be noted that variable counts were obtained for each household. Most of the houses yielded mosquitos in their vicinity (outside) with counts of 0–50 mosquito individuals (i.e., larvae, pupa, and adult). A moderate number of houses yielded counts of 50–100 mosquito individuals and a few houses with 100–150 counts across the different collection times. There were collection times where these counts were exceeded; and in Area 1- Old Balara, in particular, there were two instances, where it had counts exceeding 150 specimens per collection, followed by Area 4- Sitio Payong and lastly by Area 2- Luzon. This result was correlated with the cleanup program of the community and the prevalence of dengue cases.

Mosquito samples were submitted to the Research Institute for Tropical Medicine (RITM), Dept of Medical Entomology, for taxonomic identification. The results showed a 100% match for the preliminary identification in the lab and those identified in RITM, where most of the samples are *A. aegypti* and a few are *A. albopictus* and *Culex sp*. (See **Figure A1**).

A coordination meeting with RITM was conducted toward organizing a dengue study group. The RITM Virology Lab shared a protocol to detect dengue by RT-PCR. This procedure was optimized to detect the dengue virus in mosquitoes and was used in the analysis of the specimens collected from the different communities, including those submitted by the Philippine Nuclear Research Institute (PNRI, which were reared in the PNRI Mosquito laboratory for several generations) (**Figure 5**). Rearing of *Ae. aegypti* larvae were done using deionized water and commercial fish meal (Tetramin, Tetra GmbH) at 0.02 mg/larva/day. Pupae are collected as soon as they develop. Adults were confined in a rearing cage (1 ft^3) and fed with a 10% sugar solution. Adult females were blood-fed using immobilized live mice. Egg collection was done using an egging cup (40 mL cap.), containing about 10 mL deionized water and lined with white filter paper for oviposition. *Ae. aegypti* was reared in laboratory conditions with a mean temperature of 27°C, relative humidity of 70%, and photoperiod of 12:12 (light: dark).

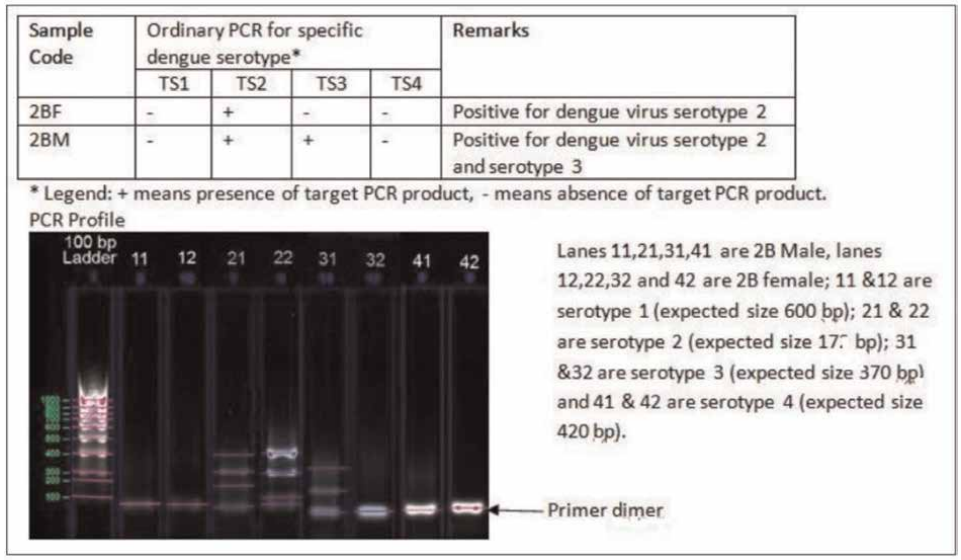


Figure 5.
Results of selected mosquito samples analyzed for the 4 serotypes. Lanes 21 and 31 indicate the presence of serotype 2 and 3 in the same male Aedes *sample and only serotype 2 (lane 22) for the female sample. Note the multiple bands, indicating multiple genotypes for each serotype.*

Initial results indicated that 8 out of 10 specimens are dengue positive, with both male and female mosquitos showing positive results for the dengue virus infection. These infections could be vertically transmitted (maternal to offspring) as the samples were obtained from hatched larvae that were reared to the adult stage (**Figure 6**). The same pattern was observed in the samples collected from the traps set up in Barangay Old Balara where larvae were made to hatch, and the emerging adult mosquitos were analyzed for the presence of the dengue virus. Results showed that multiple dengue serotypes could infect one mosquito and multiple genotypes within a serotype can also occur (**Figure 5**). Vertical transmission has been reported to occur among dengue viruses in the *Aedes* mosquito [30–32]. The findings of this study are consistent with these previous reports.

In the second year of genomic surveillance in the community, eleven sampling events were established and monitored. The mosquito collection from the first to the eleventh collection was processed, recorded, and summarized as shown in **Figure 7**. It was seen from the trend that there was a persistent prevalence of mosquitos in Area 2 followed by Area 4 and least by Area 1. As expected, most collections were larvae and pupae with most samples coming from Area 2, followed by Area 4, and lastly by Area 1 (**Figure 6**).

Areas 1 and 4 are more forested communities as compared to Area 2, which contrasts with the expected pattern that *A. aegypti* tends to prefer forested areas. The low trapping yield in Area 1 could also reflect on the anti-dengue mosquito program of the community as the community health center is located in Area 1.

It can also be noted that the pattern where the majority of the strongly dengue-positive mosquitos were those collected in the second to the sixth collection, which were from August to November and began to decline in the seventh to the eleventh collection, which was from December to February. Most of the strongly dengue-positive specimens were found in Areas 2 and 4. These findings on the dengue PCR

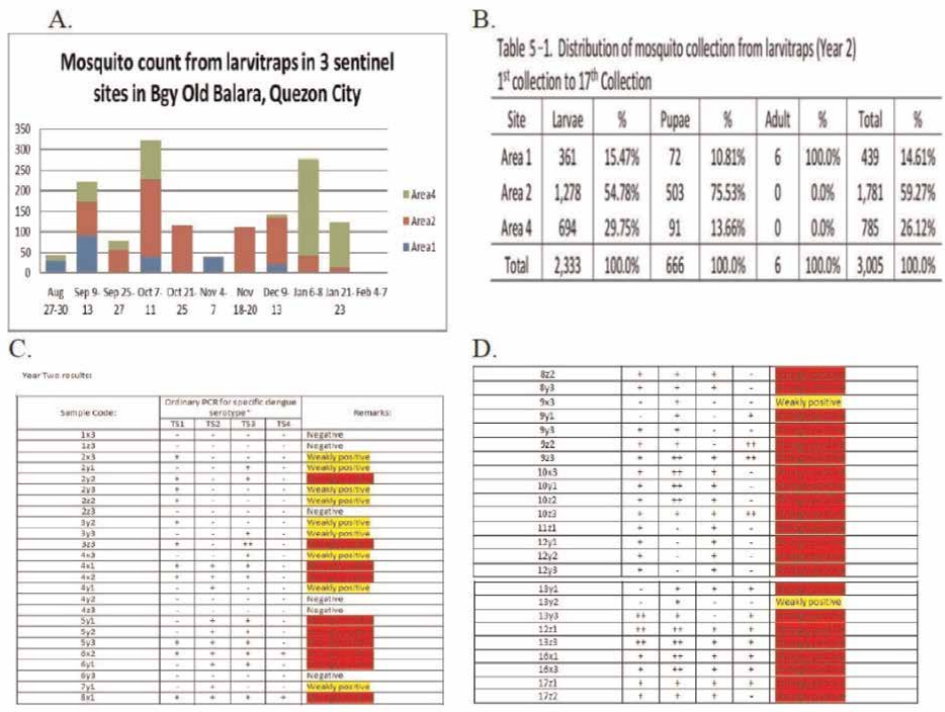


Figure 6.
Distribution summary of mosquitoes collected from the three sentinel sites in Bgy Old Balara. Area 1 (Old Balara), Area 2 (Luzon), and Area 4 (Sitio Payong) in year 2. Firstto eleventhcollection (A). Table 5-1 summarize the counts of specimens collected (B). Dengue-PCR results of the specimens collected and scoring best on the degree of dengue positivity (C-D) with red meaning strongly positive and yellow as weakly positive.

pattern tend to support the collection data in **Figure 6**. This means that vector surveillance with accompanying RT-PCR detection of the dengue virus serotypes can provide an additional layer of information that would reflect the seasonal variations of dengue infestation of the mosquito vector as well as the dengue management program of the community.

3.2 Development of antisense RNA for dengue virus in mosquito

The RNAi protocol developed was implemented, and was able to yield antisense RNA products for testing on mosquitos. Two trials were conducted on RNAi-based inhibition of the dengue virus in the *Aedes* mosquito derived from Barangay. Old Balara is shown in **Figure 7**. Evaluation of a mosquito feeding device for RNAi construct delivery system was not pursued as direct brown sugar feeding was found to be a simple strategy considering that RNA is stable in a sugar solution. Apparently, the simpler the delivery system is, the better it will be for community-based intervention. In the result shown in **Figure 7**, we can see that the feeding of antisense RNA in 4–7 days resulted in the shearing of dengue viral RNA. The mosquitos mostly die on the fourth day, indicating the lethality of the antisense RNA. It was also observed that those mosquitos that survived on the 7th day showed clearance from all serotypes.

The test was repeated to evaluate which of the following antisense RNAs will best work: dsRNA non-hybridized, dsRNA hybridized, ssRNA+ strand, or ssRNA-strand. The results of this experiment are shown in **Figures 7** and **3**. Only the dsRNA hybridized

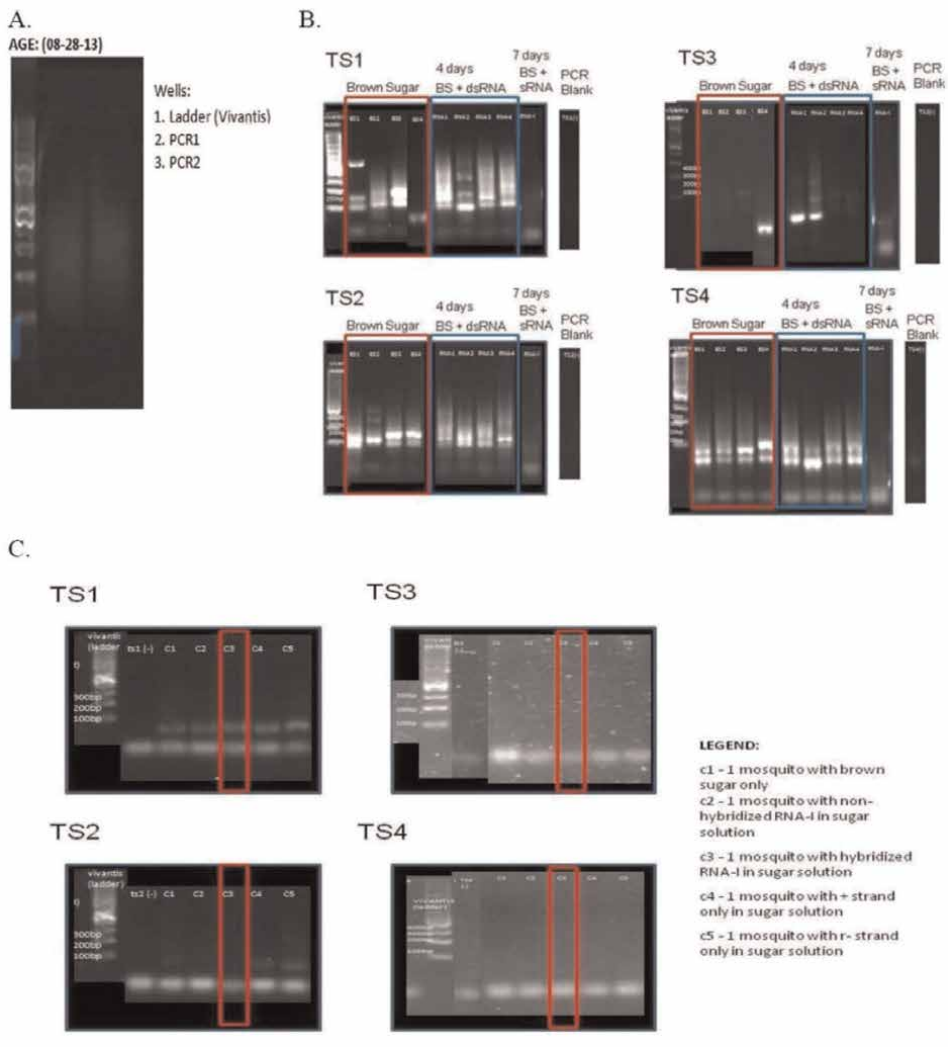
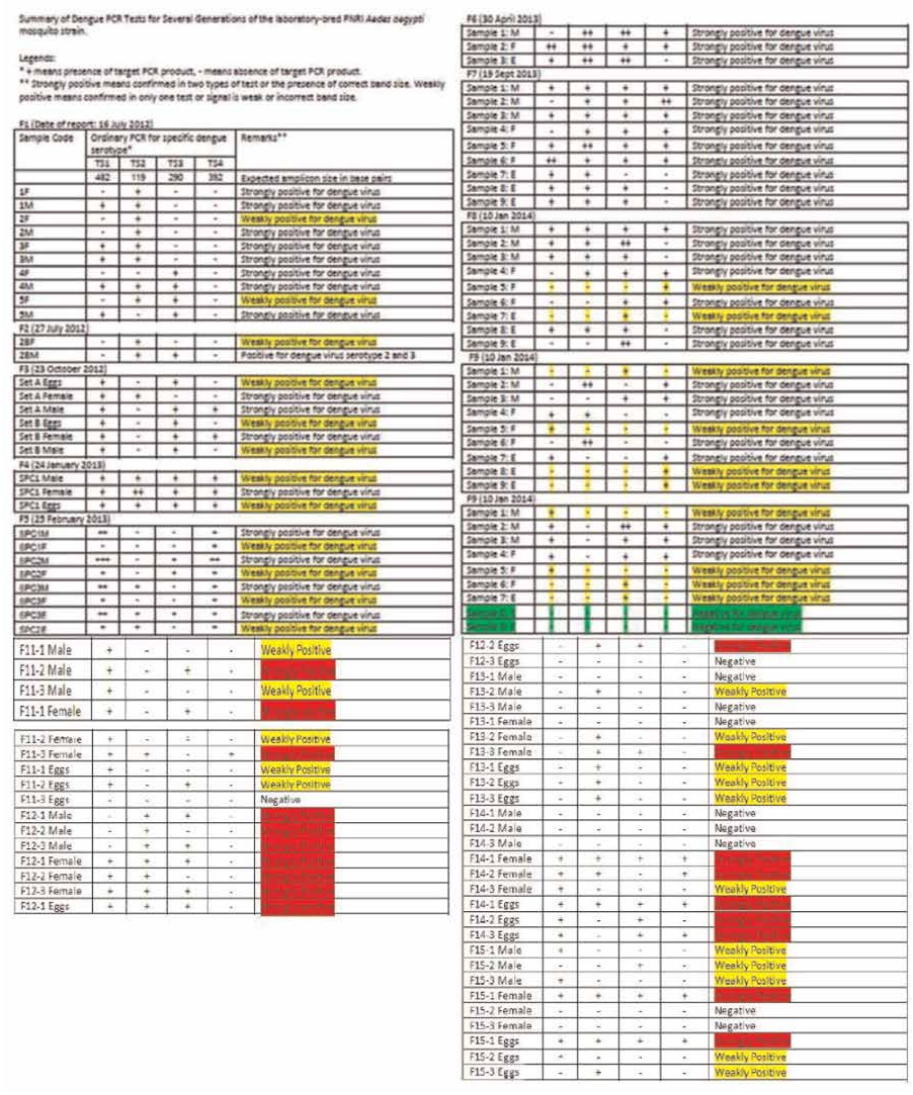


Figure 7.
Antisense RNA synthesis and trial testing on mosquitoes. A. Preliminary antisense RNA synthesis results. B. Results on antisense RNA representing 4 days and 7 days feeding with RNAi. TS1-TS4 represents the four dengue serotypes. Notable is the disintegration of signals in 4 days (i.e., shearing of DNA) as compared to brown sugar and 7 days (i.e., faint shearing of DNA). The antisense produced also affords protection of different serotypes. C. Trial 3 results in antisense RNA representing 4 days feeding with RNAi and comparison of different RNAi types. Notable is the specific disintegration of signals in TS2 but not TS1 as compared to brown sugar only. The lowest bands are primer dimers.

showed activity in the specific inhibition of TS2. There is an endogenous reduction in TS2 dengue signal with sugar alone, while intensification of signal with either ssRNA+ strand or ssRNA-strand was observed. In this sample, no serotype 3 or 4 was present. It can be noted that TS1 is not inhibited when the designed RNAi is generic.

3.3 Vertical transmission of dengue in in-house propagated mosquito stocks

Specimens from F1 to F15 generation obtained from the PNRI were analyzed with dengue PCR results shown in **Figure 8**. It can be observed that a persistent occurrence



Summary of Dengue PCR Tests for Several Generations of the laboratory-bred PNRI *Aedes aegypti* mosquito strain.

Legends:
* + means presence of target PCR product, - means absence of target PCR product.
** Strongly positive means confirmed in two types of test or the presence of correct band size. Weakly positive means confirmed in only one test or signal is weak or incorrect band size.

F1 (Date of report: 16 July 2012)

Sample Code	Ordinary PCR for specific dengue serotype*				Remarks**
	TS1	TS2	TS3	TS4	
	482	119	290	392	Expected amplicon size in base pairs
1F	-	+	-	-	Strongly positive for dengue virus
1M	+	+	-	-	Strongly positive for dengue virus
2F	-	+	-	-	Weakly positive for dengue virus
2M	-	+	-	-	Strongly positive for dengue virus
3F	+	+	-	-	Strongly positive for dengue virus
3M	+	+	-	-	Strongly positive for dengue virus
4F	-	-	+	-	Strongly positive for dengue virus
4M	+	+	+	-	Strongly positive for dengue virus
5F	-	+	+	-	Weakly positive for dengue virus
5M	+	-	+	-	Strongly positive for dengue virus

F2 (27 July 2012)

2BF	-	+	-	-	Weakly positive for dengue virus
2BM	-	+	+	-	Positive for dengue virus serotype 2 and 3

F3 (23 October 2012)

Set A Eggs	+	-	+	-	Weakly positive for dengue virus
Set A Female	+	+	-	-	Strongly positive for dengue virus
Set A Male	+	-	+	+	Strongly positive for dengue virus
Set B Eggs	+	-	+	-	Weakly positive for dengue virus
Set B Female	+	-	+	+	Strongly positive for dengue virus
Set B Male	+	-	+	-	Weakly positive for dengue virus

F4 (24 January 2013)

SPC1 Male	+	+	+	+	Weakly positive for dengue virus
SPC1 Female	+	++	+	+	Strongly positive for dengue virus
SPC1 Eggs	+	+	+	+	Weakly positive for dengue virus

F5 (25 February 2013)

SPC1M	**	-	-	+	Strongly positive for dengue virus
SPC1F	-	+	-	+	Weakly positive for dengue virus
SPC2M	***	-	+	**	Strongly positive for dengue virus
SPC2F	+	-	+	+	Weakly positive for dengue virus
SPC3M	**	+	-	+	Strongly positive for dengue virus
SPC3F	+	-	-	+	Weakly positive for dengue virus
SPC3E	**	+	+	+	Strongly positive for dengue virus
SPC2E	+	+	-	+	Weakly positive for dengue virus

F11-1 Male	+	-	-	-	Weakly Positive
F11-2 Male	+	-	+	-	
F11-3 Male	+	-	-	-	Weakly Positive
F11-1 Female	+	-	+	-	

F11-2 Female	+	-	-	-	Weakly Positive
F11-3 Female	+	+	-	+	
F11-1 Eggs	+	-	-	-	Weakly Positive
F11-2 Eggs	+	-	+	-	Weakly Positive
F11-3 Eggs	-	-	-	-	Negative
F12-1 Male	-	+	+	-	
F12-2 Male	-	+	-	-	
F12-3 Male	-	+	+	-	
F12-1 Female	+	+	+	-	
F12-2 Female	+	+	-	-	
F12-3 Female	+	+	+	-	
F12-1 Eggs	+	+	+	-	

F6 (30 April 2013)

Sample 1: M	-	++	++	+	Strongly positive for dengue virus
Sample 2: F	++	++	+	+	Strongly positive for dengue virus
Sample 3: E	+	++	++	-	Strongly positive for dengue virus

F7 (19 Sept 2013)

Sample 1: M	+	+	+	+	Strongly positive for dengue virus
Sample 2: M	-	+	+	++	Strongly positive for dengue virus
Sample 3: M	+	+	+	+	Strongly positive for dengue virus
Sample 4: F	-	+	+	+	Strongly positive for dengue virus
Sample 5: F	+	++	+	+	Strongly positive for dengue virus
Sample 6: F	++	+	+	+	Strongly positive for dengue virus
Sample 7: E	+	+	-	-	Strongly positive for dengue virus
Sample 8: E	+	+	+	-	Strongly positive for dengue virus
Sample 9: E	+	+	+	-	Strongly positive for dengue virus

F8 (10 Jan 2014)

Sample 1: M	+	+	+	+	Strongly positive for dengue virus
Sample 2: M	+	+	++	-	Strongly positive for dengue virus
Sample 3: M	+	+	+	-	Strongly positive for dengue virus
Sample 4: F	-	+	+	+	Strongly positive for dengue virus
Sample 5: F	+	+	+	+	Weakly positive for dengue virus
Sample 6: E	-	-	+	+	Strongly positive for dengue virus
Sample 7: E	+	+	+	+	Weakly positive for dengue virus
Sample 8: E	+	+	+	-	Strongly positive for dengue virus
Sample 9: E	-	-	++	-	Strongly positive for dengue virus

F9 (10 Jan 2014)

Sample 1: M	+	+	+	+	Weakly positive for dengue virus
Sample 2: M	-	++	-	+	Strongly positive for dengue virus
Sample 3: M	-	-	+	+	Strongly positive for dengue virus
Sample 4: F	+	+	-	-	Strongly positive for dengue virus
Sample 5: F	+	+	+	+	Weakly positive for dengue virus
Sample 6: F	-	++	-	-	Strongly positive for dengue virus
Sample 7: E	+	-	-	+	Strongly positive for dengue virus
Sample 8: E	+	+	+	+	Weakly positive for dengue virus
Sample 9: E	+	+	+	+	Weakly positive for dengue virus

F10 (10 Jan 2014)

Sample 1: M	+	+	+	+	Weakly positive for dengue virus
Sample 2: M	+	-	++	+	Strongly positive for dengue virus
Sample 3: M	+	-	+	+	Strongly positive for dengue virus
Sample 4: F	+	-	+	+	Strongly positive for dengue virus
Sample 5: F	+	+	+	+	Weakly positive for dengue virus
Sample 6: F	+	+	+	+	Weakly positive for dengue virus
Sample 7: E	+	+	+	+	Weakly positive for dengue virus
Sample 8: E					
Sample 9: E					

F12-2 Eggs	-	+	+	-	
F12-3 Eggs	-	-	-	-	Negative
F13-1 Male	-	-	-	-	Negative
F13-2 Male	-	+	-	-	Weakly Positive
F13-3 Male	-	-	-	-	Negative
F13-1 Female	-	-	-	-	Negative
F13-2 Female	-	+	-	-	Weakly Positive
F13-3 Female	-	+	+	-	
F13-1 Eggs	-	+	-	-	Weakly Positive
F13-2 Eggs	-	+	-	-	Weakly Positive
F13-3 Eggs	-	+	-	-	Weakly Positive
F14-1 Male	-	-	-	-	Negative
F14-2 Male	-	-	-	-	Negative
F14-3 Male	-	-	-	-	Negative
F14-1 Female	+	+	+	+	
F14-2 Female	+	+	-	+	
F14-3 Female	+	-	-	-	Weakly Positive
F14-1 Eggs	+	+	+	+	
F14-2 Eggs	+	-	+	-	
F14-3 Eggs	+	-	+	+	
F15-1 Male	+	-	-	-	Weakly Positive
F15-2 Male	-	-	+	-	Weakly Positive
F15-3 Male	+	-	-	-	Weakly Positive
F15-1 Female	+	+	+	+	
F15-2 Female	-	-	-	-	Negative
F15-3 Female	-	-	-	-	Negative
F15-1 Eggs	+	+	+	+	
F15-2 Eggs	-	-	-	-	Weakly Positive
F15-3 Eggs	-	+	-	-	Weakly Positive

Figure 8.
Summary dengue PCR results of F1 to F15 generation of mosquito samples in-house bred in PNRI. Dengue-free eggs emerged in F10 as shown in green. Dengue-positive mosquitoes are shown in yellow (i.e., weakly positive) and red (i.e., strongly positive).

of the dengue virus from F1 to F10 samples (variable pattern could arise from a random sampling of 3 M/F/E samples). We observed the presence of different serotypes in one mosquito in females, males, and egg samples. We also observed the presence of different genotypes in one serotype (multiple bands were verified by sequencing, which will be reported in another paper). A yield of dengue-free eggs was observed in F10. Thus, the transovarial transmission of the dengue virus in local *A. aegypti* mosquitos has been verified. We also analyzed a batch sample of mosquito eggs consisting of 20 eggs. The dengue PCR results show only four eggs positive with serotype 1 and none for all the other serotypes (See **Figure A2**). This shows that the infection rate for vertically transmitted dengue virus template from parent to egg is approximately 20%.

Whether the amplicons detected through this dengue-specific PCR represent authentically, and live viruses may require definitive proof by DNA sequencing. Such will be presented in another paper.

A study on transovarial transmission of dengue in correlation with virulence in mice was conducted by our graduate student (i.e., Mr. Ralph Bawalan—MS Trop Med) in collaboration with Dr. Nelia Salazar—RITM Entomologist. A mouse model for dengue testing was developed. This model was able to show histological similarities with humans as well as pathological symptoms of thrombocytopenia and fever (See **Figure A3**).

3.4 Genomic surveillance in year 3 and strategy for mitigation

The genomic surveillance was continued for Area 1-Old Balara, Area 2-Luzon, and Area 4-Sitio Payong through their sentinel sites. The mosquito/larvae/pupae collected from the sites from first to the nineth collection and their respective dengue PCR results are summarized as shown in **Figure 9**. It can be observed that at all sites, there

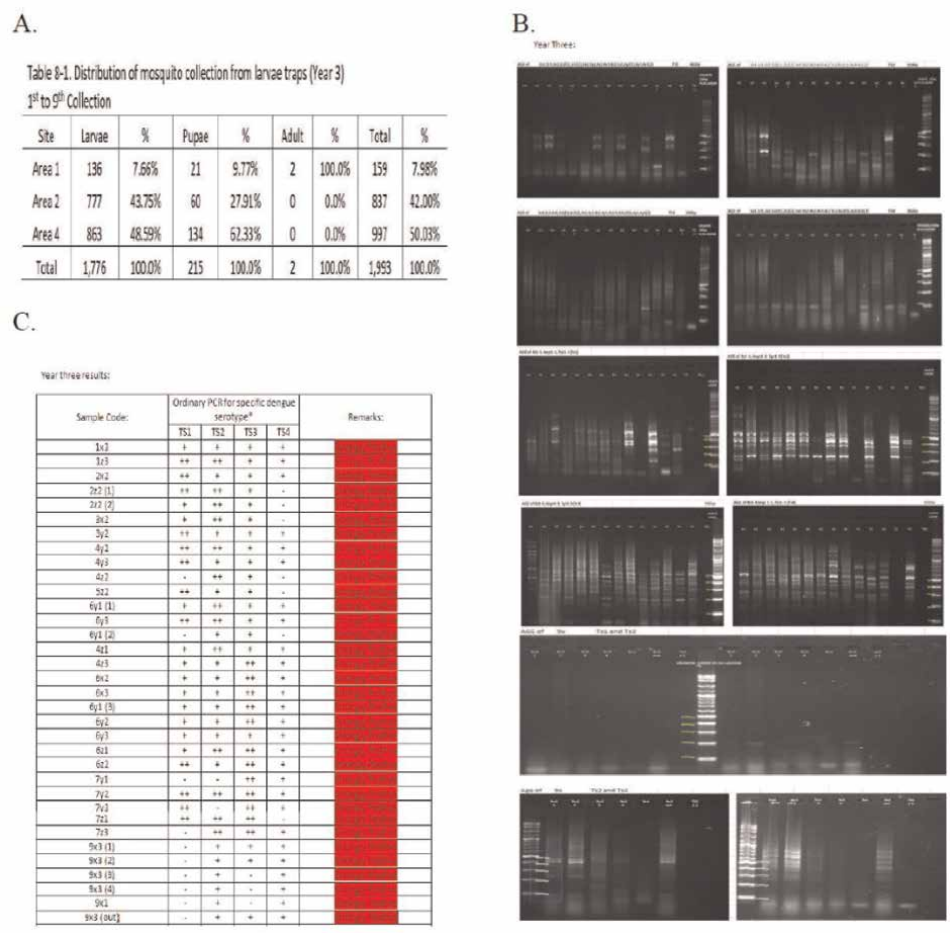

A.

Table 8-1. Distribution of mosquito collection from larvae traps (Year 3)
1st to 9th Collection

Site	Larvae	%	Pupae	%	Adult	%	Total	%
Area 1	136	7.66%	21	9.77%	2	100.0%	159	7.98%
Area 2	777	43.75%	60	27.91%	0	0.0%	837	42.00%
Area 4	863	48.59%	134	62.33%	0	0.0%	997	50.03%
Total	1,776	100.0%	215	100.0%	2	100.0%	1,993	100.0%

C.

Year three results:

Sample Code:	Ordinary PCR for specific dengue serotype*				Remarks:
	TS1	TS2	TS3	TS4	
1x2	+	+	+	+	
1z3	++	++	+	+	
2x2	++	+	+	+	
2z2 (1)	++	++	+	-	
2z2 (2)	+	++	+	-	
8x2	+	++	+	-	
3y2	++	+	+	+	
4y2	++	++	+	+	
4y3	++	+	+	+	
4z2	-	++	+	-	
5z2	++	+	+	-	
6y1 (1)	+	++	+	+	
6y3	++	++	+	+	
6y1 (2)	-	+	+	-	
4y1	+	++	+	+	
4y3	+	+	++	+	
6x2	+	+	++	+	
6x3	+	+	++	+	
6y1 (3)	+	+	++	+	
6y2	+	+	++	+	
6y3	+	+	+	+	
6z1	+	++	++	+	
6z2	++	+	++	+	
7y1	-	-	++	+	
7y2	++	++	++	+	
7y3	++	-	++	+	
7z1	++	++	++	-	
7z3	-	++	++	+	
9x3 (1)	-	+	+	+	
9x3 (2)	-	+	+	+	
9x3 (3)	-	+	-	+	
9x3 (4)	-	+	-	+	
9C1	-	+	-	+	
9x3 (out)	-	+	+	+	

B.

Year Three:

Figure 9.
Distribution summary of the genomic surveillance in year 3. A. Table 8-1 contains the consolidated mosquito specimens collected from the different sites. B. Dengue PCR amplicon electropherograms of community specimens. C. Dengue-PCR results of the specimens collected and scoring based on the degree of dengue positivity with red meaning strongly positive.

was a strong positivity for the dengue virus, indicating a worsening dengue infection from the community mosquito population after 3 years of surveillance. Apparently, the 4S strategy of the community does not seem to work sustainably in containing the spread of the virus. How this correlates with the dengue disease burden of the community may have to be closely evaluated.

The remaining mosquito larvae' homogenates and extracts were evaluated for their potential use as templates for the antisense experiments. The plan was to amplify the dengue virions that can be recovered from intra-cranial injections in suckling mice, a procedure that was previously optimized by our team (i.e., based on **Figure A3**).

A community trial of the antisense RNA formulation was installed in Area 1 (Old Balara) and Area 4 (Sitio Payong). The fourth and sixth batches of collected samples that are positive for dengue virus (serotypes 1 to 4) were utilized in the experiment (**Figure 3**).

After 2 weeks of exposure, specimens were collected, extracted, and tested for dengue-PCR. The results showed low to the absence of signals in antisense RNAi-fed.

specimens as compared to those that were not fed. The results are consistent with the previous findings. Even in the community environment, the antisense RNA preparation was able to inhibit the dengue virus transmission in the mosquito (**Figure 3**). It was also noted that in an ovi-larvae trap model, there was inhibition of the dengue virus, which is carried over to the emerging mosquito. This is a new indication of the developed antisense RNA.

4. Discussion

Since 2012, three studies have been conducted in the Philippines that verified and validated the natural vertical transmission of the dengue virus in a community population [12–14]. Similarly, there were seasonal variations in dengue positivity in the mosquito, mostly occurring in the rainy seasons of August to November, which is aggravated by possibilities of multiple serotypes and multiple genotype patterns, indicating that the next generation of eggs laid by a dengue-infected mosquito become the melting pot for possible dengue virus recombination. Apparently, the data also indicate that the *A. aegypti* mosquito has been successfully adapting, breeding, and successfully thriving as a coexisting dengue vector in urban communities and the absence of forested areas no longer limits its geographical spread. Through the years, while the *Aedes* mosquito has continued to infiltrate the urban communities, the mitigation strategy implemented by the Department of Health to all the Barangay communities has not taken major leaps and still follows the 4S Program (DOH website). Thus, updated and leveled-up interventions may have to be implemented and integrated into community-based strategies to be able to see concrete progress in dengue intervention. As outlined in the conceptual framework in **Figure 1B**, the genomic surveillance strategy for the dengue virus harbored by the natural stocks of mosquitos captured from ovi-traps in sentinel sites in the community may have to be set up in coordination with the barangay health center and neighboring academic or coordinating research institutions with existing PCR facilities for routine molecular detection followed by online reporting of results to allow quick action of the community to implement various interventions. Furthermore, given the knowledge of the circulating dengue serotypes and genotypes in the mosquito community population and their potential to persist in the next generation of mosquitos, various genomic-based interventions may be designed and implemented, among them are the sterile

insect technique, which introduces noninfected, sterile male mosquitos that will breed in natural stocks and control the egg-laying potential, thus gradually controlling the natural mosquito population. The other approach is through this *Wolbachia sp*. infection, a natural bacterium selectively growing in *Aedes* mosquitoes and would result in the eventual death of the infected mosquitos, thereby reducing the natural population. Another approach, which is done in this study is by antisense RNA, which can be designed based on the circulating variant of the virus, the templates of which are derived from the genomic surveillance DNA/RNA extracts, and are introduced or actively fed to mosquitos in the communities to block the vertical transmission of the circulating dengue viruses. Since it is not entirely possible to eliminate the mosquito population, the antisense RNA can be designed to not only block the dengue vertical transmission but also provide gene-targeting strategies that would reduce reproductive capacities, including among others egg-laying or hatching potentials. The feeding platform may involve simple technologies readily adaptable to communities such as the brown sugar solution used for feeding insects, such as butterflies, which may be enhanced with lactic acid or blood meal to promote mosquito consumption of the antisense RNA formula. The challenge; however, in this approach is assessing the long-term safety and efficacy of double-stranded RNAs and their effect in reshaping the patterns and demographic structure of the dengue virus in the natural mosquito population. The health benefits to humans though may outweigh the ecological impact of this type of mitigation.

5. Conclusions and recommendations

The genomic surveillance from year 1 of the three areas in Barangay Old Balara, an urbanized area showed an increasing trend of more than 3–4 houses per site (20–27%) that are found to be positive for dengue. A website established to report results of the genomic surveillance found utility for the online access of the Barangay health workers and provided support for their search and destroy program against the breeding ground of mosquitos. Species identification of the community-collected specimens indicated the majority to be *A. aegypti* and a few *A. albopictus* and *Culex sp*. The RT-PCR surveillance revealed the presence of multiple dengue serotypes in one mosquito specimen and multiple genotypes within a serotype. There was a persistent prevalence of mosquitos in Area 2 followed by Areas 4 and 1, considering that Area 2 was less forested, which contrasts with the expected pattern for *A. aegypti,* which tends to prefer forested areas. The low trapping yield in Area 1 reflects the anti-dengue mosquito program of the community as the community health center is in Area 1. Strong dengue positivity was found in mosquitos collected in the second to the sixth collection, which was from August to November and declined on the seventh to the eleventh collection, which was from December to February. This indicates that the vector surveillance with accompanying RT-PCR detection of the dengue virus serotypes can provide an additional layer of information that would reflect the seasonal variations of dengue infestation of the mosquito vector as well as the possible congruence of the dengue management program of the community.

The antisense RNA preparation that was developed based on the dengue amplicons obtained from the genomic surveillance was able to inhibit the dengue virus transmission in the mosquito from one generation to the other in a simulated community setting. Further studies can be done to evaluate the potential utility of a genomic surveillance-based antisense RNA platform in real-life community scenarios. While

vertical transmission of dengue has been established as a known mechanism for the persistent presence of dengue in *Aedes* mosquito populations found in the communities, the current 4S strategy implemented locally may not be adequate to control the rising dengue cases and an active genomic-based intervention to block this vertical transmission must be done.

Acknowledgements

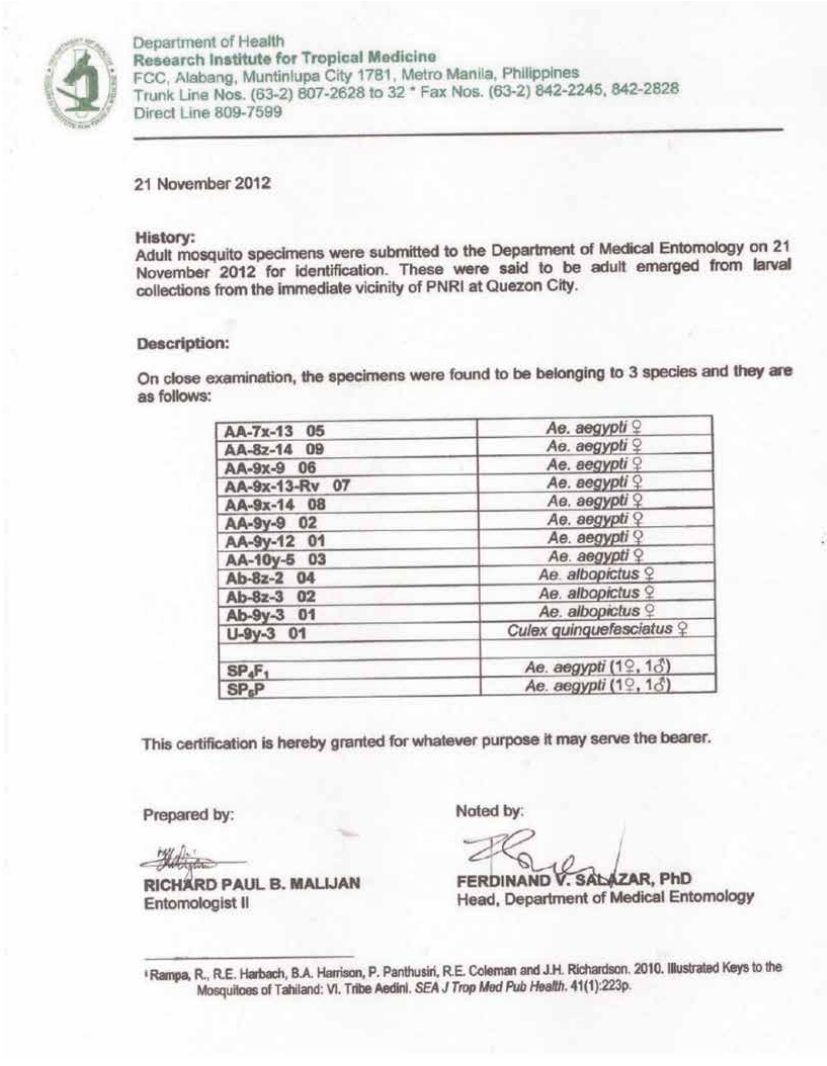
Special thanks to the National Research Council of the Philippines (NRCP) for the research grant of FMH (NRCP Grant # N-001). Also, thanks to the assistance and support of the Quezon City Health Department, particularly Dr. Antonieta Inumerable and Dr. Rolly Cruz; and the officials of Barangay Old Balara, Quezon City, particularly Hon. Beda Torrecampo and Dra. Karen Alcid-See. Special thanks to Dr. Cecilia Reyes, Entomologist and former Director of NRCP who engaged our team in this project, Dr., Lourdes J. Cruz, National Scientist and former President of NRCP, all the NRCP management and staff who help us through the years, especially Ms. Renia Corocoto and Mr. Caezar Arceo, and the other members of the ReM0VE Dengue Program, Dr. Grace Yu, Dr. Nelia Salazar, Dr. Judylin Solidum, Dr., Pio Javier, and Dr. Erlinda Torres.

Special thanks also to Ralph Bawalan, former MS Trop Medicine student who worked with us and now pursuing his Ph.D., and his adviser, Dr. Nelia Salazar.

Conflict of interest

The authors declare no conflict of interest.

Appendix



Department of Health
Research Institute for Tropical Medicine
FCC, Alabang, Muntinlupa City 1781, Metro Manila, Philippines
Trunk Line Nos. (63-2) 807-2628 to 32 * Fax Nos. (63-2) 842-2245, 842-2828
Direct Line 809-7599

21 November 2012

History:
Adult mosquito specimens were submitted to the Department of Medical Entomology on 21 November 2012 for identification. These were said to be adult emerged from larval collections from the immediate vicinity of PNRI at Quezon City.

Description:

On close examination, the specimens were found to be belonging to 3 species and they are as follows:

AA-7x-13 05	*Ae. aegypti* ♀
AA-8z-14 09	*Ae. aegypti* ♀
AA-9x-9 06	*Ae. aegypti* ♀
AA-9x-13-Rv 07	*Ae. aegypti* ♀
AA-9x-14 08	*Ae. aegypti* ♀
AA-9y-9 02	*Ae. aegypti* ♀
AA-9y-12 01	*Ae. aegypti* ♀
AA-10y-5 03	*Ae. aegypti* ♀
Ab-8z-2 04	*Ae. albopictus* ♀
Ab-8z-3 02	*Ae. albopictus* ♀
Ab-9y-3 01	*Ae. albopictus* ♀
U-9y-3 01	*Culex quinquefasciatus* ♀
SP_4F_1	*Ae. aegypti* (1♀, 1♂)
SP_8P	*Ae. aegypti* (1♀, 1♂)

This certification is hereby granted for whatever purpose it may serve the bearer.

Prepared by:

RICHARD PAUL B. MALIJAN
Entomologist II

Noted by:

FERDINAND V. SALAZAR, PhD
Head, Department of Medical Entomology

[1] Rampa, R., R.E. Harbach, B.A. Harrison, P. Panthusiri, R.E. Coleman and J.H. Richardson. 2010. Illustrated Keys to the Mosquitoes of Tahiland: VI. Tribe Aedini. *SEA J Trop Med Pub Health*. 41(1):223p.

Figure A1.
Entomological report certifying the taxonomic identity of the collected mosquito from the community.

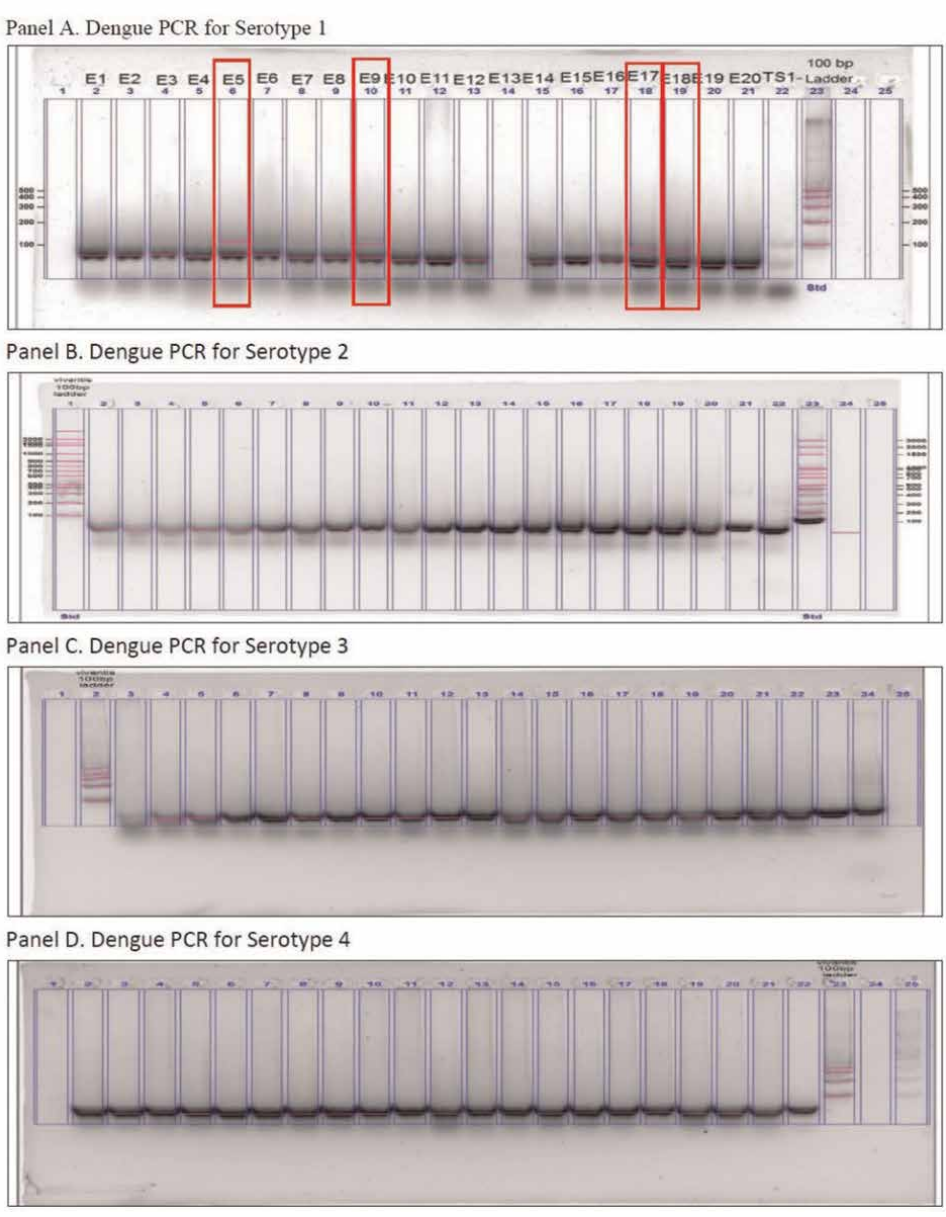


Figure A2.
Electropherogram of the PCR products of 20 mosquito eggs from F10 generation showing approximately 20% infection rate.

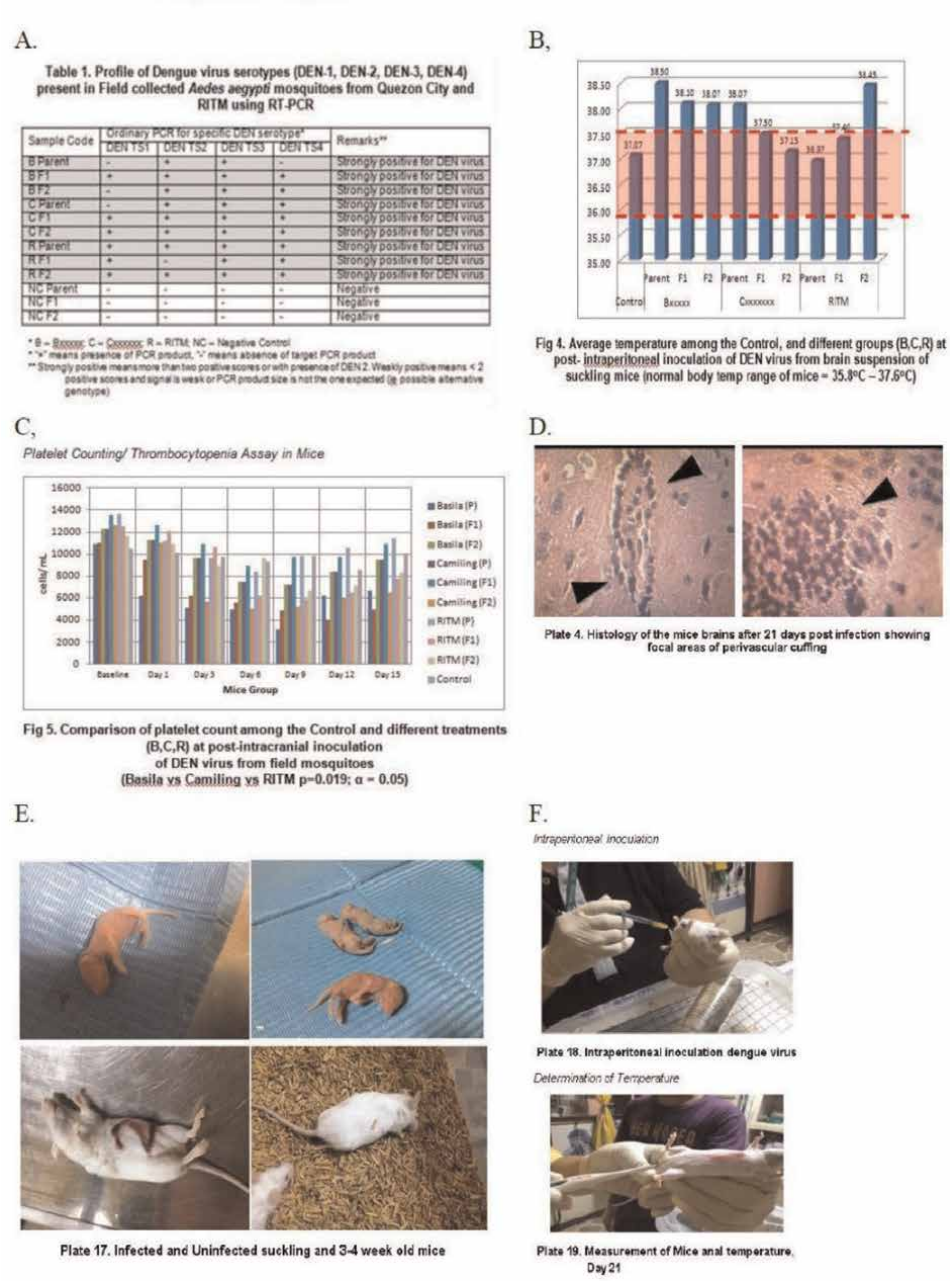


Figure A3.
Viral expansion through neonate intra-cranial injection of RNA extracts from community-collected mosquito specimens and virulence assay in mice of the subsequent generation.

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Chapter 14

Dengue Reduction through Vector Control

Eduardo A. Fernandez Cerna, Catalina Sherman and Mercedes Marlene Martinez

Abstract

Dengue fever is a disease transmitted by the mosquito *aegypti*. There is a secondary vector: Aedes albopictus with some epidemiological importance in the transmission of dengue. Pharmacological treatment for dengue is a palliative treatment for the disease and there is an absence of a universally accepted vaccine for the different clinical infections. In these circumstances, the interruption of the infection cycle is possible basically through the reduction of the *Aedes aegypti*, reducing its breeding sites or physically reducing its population through chemical or biological means. Traditional approaches to vector control are becoming less effective as a result of the combination of resistance to insecticides and the logistic complexity of covering increasingly large urban centers with the same number of health workers as in past decades. Experiences in different countries reflect the need to involve more actively families and communities in the reduction of breeding sites. Several innovations have been introduced using biological methods, physical control of sources, and involvement of families and schools in vector control. The possibility to scale up successful experiences requires a joint effort of governments and communities to tackle mosquito source reduction and add a multipurpose concept of domestic hygiene.

Keywords: dengue, *Aedes aegypti*, breeding sites, control methods, community, hygiene

1. Introduction

Dengue fever is a viral infection transmitted by a mosquito. Different studies calculate that 3900 million people are living at risk of contracting a dengue infection. According to model-based estimations every year, there are 390 million infections caused by the dengue virus, and from those 96 million present clinical manifestations ranging from very mild to extremely severe and life threatening [1].

Dengue fever is endemic of tropical and subtropical regions where weather conditions favor the presence of its vector *A. aegypti* mosquitoes and its alternative vector *Aedes albopictus* with more prevalence in Asia but is now also present in the Americas. In these regions, the contrasting conditions of rainfall and severe lack of accessible water supply enable the presence of breeding sites in artificial containers filled by the rainfall during the rainy season and water reservoirs kept by the population to assure its access during the severe dry season (tropical summer) [2, 3]. Interestingly enough,

natural conditions and human behaviors induced by the need to have access to reliable water supply are the catalyzers to the presence of high densities of *A. aegypti* mosquitoes and as a consequence the transmission of dengue fever [4], which is explained in its clinical characteristic elsewhere in this book.

Dengue is caused by a family of viruses (flavivirus) that are carried by the *A. aegypti* females from infected human hosts to healthy but susceptible human hosts (ready to be infected) that establish a cycle of human-mosquito-human that repeats constantly keeping the viral activity and its transmission in the population [2, 3].

The transmission cycle cannot be interrupted by curative drugs (to reduce the number of cases of dengue infection), prevention through vaccines has been tried but after unsuccessful attempts to introduce effective vaccines, the pharmaceutical industry continues to work in safe vaccines without definite results [5].

The only alternative currently, as was the case a century ago, is to interrupt the transmission by reducing the population of *A. aegypti* through the elimination of the adults and immature forms (eggs, larvae, and pupae) or by making an effort to reduce the breeding sites through improving sanitation measures in human dwellings and peridomestic areas. In the 1920s, there was an extraordinary effort to eliminate the vector *A. aegypti* completely based on the destruction of breeding sites, the results were impressive in the areas it occurs under the leadership of Dr. Fred Sopper, but it was not complete and now, the objective is the reduction of Aedes populations rather than complete eradication [6].

In the Public Health field, there is a current tendency to integrate the different programs in more comprehensive approaches where local health actions are useful to get more objectives completed in large thematic areas such as environmental health improving the access to water, optimizing the refuse systems, and the beautification of peridomestic areas in the different neighborhoods.

Vector control in the twenty-first century requires not only a clear government commitment to this activity but a convinced and active population participating in the different required tasks.

This chapter then discusses strategies for population reduction through vector control.

2. Type of *A. aegypti* breeding sites

A. aegypti is the primary vector for dengue fever as well as responsible for the transmission of other diseases such as yellow fever, zika, and chikungunya that have produced epidemics throughout recent history. And most of this chapter will discuss about its control [1, 7].

The *A. aegypti* mosquito in its life cycle goes through immature stages to mature or adult stages (**Figure 1**).

The female mosquito is responsible for the transmission of dengue since in her need to obtain human protein to form eggs bites human hosts and feeds in their blood and at the same time inoculates the dengue virus. Once the female is fed is ready to complete the egg formation and lay in deposits with water where it can remain viable for days to months, once the egg hatches it becomes larvae for 4–5 days before evolving into pupae, and this stage is previous to the adult one that is reached in two days.

The immature stages then include eggs, larvae (instar I through 4), and pupae that are aquatics. The immature stages require deposits with water where these stages can be complete—those deposits are called breeding sites.

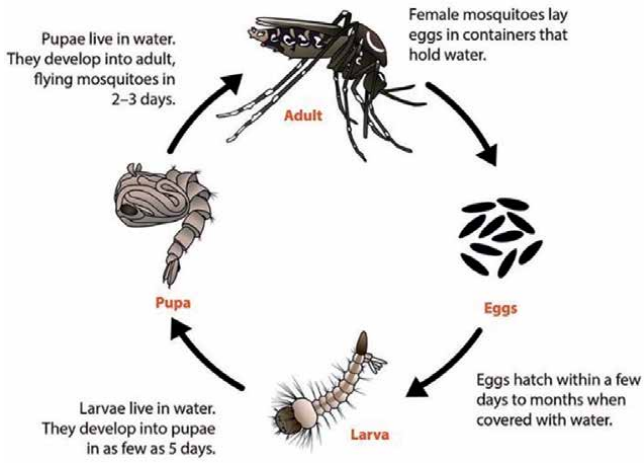

Figure 1.
Life stages of Aedes aegypti *and* Aedes albopictus *from US CDC. https://www.cdc.gov/mosquitoes/about/life-cycles/aedes.html.*

Historically, breeding sites for *A. aegypti* have been classified as: discardable and water storage containers. The discardable are usually those that are kept or thrown in peridomestic areas of the household and usually do not have an economic or utilitarian value and are the product of deficiencies in the refuse systems and/or behaviors to retain articles assuming a future use.

Discardable containers become more important during the rainy season because of their abundance in the patio; the propensity to fill with small volumes of rain and the possibility to collect water during consecutive days of rainfall. Their role as breeding sites is completed during periods of rain remissions when the female mosquitoes can lay eggs and those already in the deposits can hatch and produce the larvae forms [8, 9].

The water storage containers exist in the patios as a result of deficiencies in the regular supply of water to the households and the resulting behavior of the families to keep water collected from rain, water stream, or the water supply service when there is a chance to obtain it [9, 10]. It is worthy to mention that some discardable containers are kept in the patios with the expectation to assign a function in the future. Used tires are an example of containers with potential subjective use [11].

A third category of containers is the natural reservoirs like the leaves of some ornamental plants (bromeliads), or tree holes in the patios [8].

3. Control methods

Traditional control has been based on two strategies: reducing discardable containers and using larvicides in those water storage containers. These approaches target the vector in its immature and/or adults' stages. These methods have to do with the use of chemical products: larvicides when directed to larvae or adulticides when the mature stages are targeted.

In the last twenty years, formulations with chlorine by itself or in combination with detergents have been used in different countries in America [12, 13]. In Honduras, there were assays with sodium chlorine (kitchen salt) and limestone to modify the environment of containers like tires to make unlikely the hatching of viable eggs into larvae [14].

4. Adulticides and Larvicides

According to the World Health Organization (WHO) there are several types of vector control insecticides, divided into the following classes: organochlorines, organophosphates, carbamates, pyrethroids, bacterial larvicides, insect growth regulators and newly developed types such as neonicotinoids, spinosyns, and pyrroles [15].

Vector control programs directed to *A. aegypti* act to neutralize the adult populations using adulticides based on chemical products with an organophosphate or pyrethroid or carbamates and in the past, it was more popular to use organochlorine like DDT that has been gradually withdrawn in many countries based on environmental considerations being in use in areas of Africa and Southeast Asia with adequate results.

In the different regions, there has been an standardization of the methods to apply insecticides: residual spraying, space spraying, ITNs, treatment of nets (ITN-kits), and larviciding. Residual spraying a popular and commonly used method can be conducted indoors or outdoors. Indoor residual spraying consists of the application of residual insecticide products on indoor surfaces (e.g. walls) to kill vectors landing or resting on those surfaces; it is commonly used against indoor-resting mosquitoes [15].

Outdoor residual spraying commonly referred to as "perifocal treatment," consists of spraying the surfaces of breeding containers, with or without standing water, to obtain larvicidal and adulticidal effects on dengue vectors [15].

Space spraying, or fogging, produces insecticide-containing droplets that are small enough to remain airborne for some time [16] and are intended to cause a quick knock-down effect on flying or resting mosquito vectors upon direct contact. As low doses of chemical insecticides are used for space spraying, it does not leave a meaningful deposit that could have a residual effect [17]. Space spraying has been commonly used for the control of dengue outbreaks because of its efficacy against vector species, despite the lack of evidence of its effectiveness [15].

The use of adulticides has increased according to one of the most recent reports released by WHO for the period 2010–2019 from 434 tons in 2010 to 1680 tons in 2019 [15].

The stage of immature forms is also targeted with insecticide products generically called larvicides. The most common larvicide has been temephos, an organophosphate, but formulations with biological principles are becoming more frequent.

An interesting experience has been the use of limestone and salt in old tires laying on the patios. Tires are breeding sites that can persist positives for larvae and pupae all year long. In northern Honduras, the effect of these two products of domestic use was tested for the control of *A. aegypti* populations and the findings were highly promising, obtaining with salt a total of 112 days free of larvae and pupae, and after that time (without new treatments), a small number of larvae were produced per day (3 larvae per day compared to 15 larvae per day in control tires), which implies that with regular treatments of salt every 3 months an adequate control can be reached in these breeding sites.

When limestone (in powder) was used, the tires were kept free of larvae and pupae for 185–217 days in treated tires, and past that time without the new introduction of limestone, the production of the larval population remained minimum, which means applications of limestone can be used every 6 months to keep tires free of larvae [14].

Larvicide use of formulations based on organophosphate or pyrethroids is currently challenged by reports of resistance, and the use of salt and limestone has been mentioned as causing concerns about environmental contamination.

It is becoming more frequent to have reports of resistance to larvicides and adulticides, which represents an additional obstacle to adequate vector control [18, 19].

5. Ovicides

In a variation of the chemical control and the context of hygiene improvement, there has been a promotion of the method called little dab (la untadita) using a mixture of chlorine and detergent for the weekly cleaning of water deposits (cement tanks, cisterns, drums). This method improves the quality of deposit hygiene and has an ovicide effect on the eggs laid on the deposit walls. Days after the application of la untadita, this mixture remains with some repellent effect on the females getting to the deposits to lay eggs.

This technique was developed in 1994 based on traditional methods of cleaning water deposits but adding steps to direct the scrubbing of the walls to all surfaces covering them with a thin layer of detergent. It requires access to water to do it at least once a week and rinse it [13, 20]. See **Figure 2**.

6. Biological control

One of the prevalent ideas during several decades has been to use natural predators to destroy immature populations of *A. aegypti*. Predators such as larvivorous fishes (The biocontrol efficacy of six larvivorous fish species, namely, *Poecilia reticulata*, *Rasbora daniconius*, *Aplocheilus dayi*, *Oriochromis mossambicus*, *Oreochromis niloticus*, *Puntius bimaculatus*) and other similar species were tested in experimental conditions [21], another predator: Larvae of *Toxorrhynchites* sp. was also identified as effective at reducing larvae of *A. aegypti*. More recently *Bacillus turingiensis var israelensis* [22] and *Bacillus sphericus* (microbial agents and their spores) [23] have been also studied and found effective in experimental conditions acting as toxins for the larvae of the mosquito.

Figure 2.
Sample of a sticker for advertising the use of la Untadita. Honduras Ministry of Health.

Copepods, small crustaceans, have been identified as effective predators in different studies in the Americas and Southeast Asian countries [24].

In Honduras during the first two decades of the current century, baby turtles of the species *Trachemys scripta elegans* [25, 26] have also been used for biological control of the *A. aegypti* larvae in small-scale field research sites.

Wolbachia is endosymbiotic bacteria capable of infecting some insect species including mosquitoes causing a reproductive phenotype called cytoplasmic incompatibility having as consequence the generation of inviable offspring when uninfected females mate infected males. If the female is infected this inviability does not occur, the Wolbachia infection can continue spreading in the population. The purpose of the Wolbachia infection from a control perspective is to interfere with the transmission of the dengue virus (DENV) to *A. aegypti* [27–29].

7. Other types of control

In an experimental process, there have been experiences modifying genetically the *A. aegypti* to produce offspring which die in the first days of life, and also the release of sterile males that, mating the females, do not produce any offspring [29].

Most of the new methods are used on a small scale and are undergoing the experimental stage and require to be implemented at the national level once they are authorized for extended use.

8. Social and legal control

Some countries have opted for severe fines for households keeping uncontrolled breeding sites or obstructing the work of vector control personnel. Such is the case of Singapore and Cuba [30–32].

In both countries, transmission of dengue has been relatively low during the last decades in comparison with their neighboring countries that suffer periodical epidemics. There is value in reaching a high level of vector control but the capacity to enforce strict regulation seems more viable in a small city-state like Singapore or countries with authoritarian regimes (both examples).

However, it is important that countries have a set of regulations and enforcement measures known by the population in order to prevent the transmission and reduce the number of trespassers that require legal actions.

The vector control programs require better support from the legal system when conflicts with the population limit their function.

9. Measuring the vector control measures

For close to a century, the control measures have been assessed using traditional entomological indexes such as: Container index, House index, and Breteau indexes corresponding to the measurement of the proportion of positive deposits in a visited section of a neighborhood, a city or town or any other concentration of houses, the number of positive houses or premises in an area, and the relationship of positive deposits and supervised houses [33, 34].

Since the times of Fred Soper, there was an effort to reach indexes below 5% of positives as indicators of success, but even in highly controlled areas of Singapore with very low indexes, outbreaks of dengue have occurred [30, 31].

The limitation of the indexes is that they reflect the situation of a geographic area that is visited optimistically several times a year and in a more somber scenario once a year. They reflect the concentration of larvae in a container, which is not an accurate measurement of the potential of the breeding site to produce a healthy adult mosquito population. Different levels of mortality may depend on the container and its capacity to sustain larvae and pupae. Several studies prefer to calculate the presence of pupae because that stage does not need to feed in the container giving more predictability of the adult population to emerge from the breeding site, and they are 24 to 48 hours away from the emergences of adults that are at the active stage for the viral transmission (36). Traditional *A. aegypti* larval indices do not differentiate between containers in which all the immature stages are present and those which hold only first- and second-instar larvae. This means measuring pupae population represents an advantage [35].

A different way to measure both the activity of *A. aegypti* and the success of the vector control efforts is the installation of Ovitraps in sentinel sites to assess the oviposition activity of existing female *A. aegypti* in an area of study. The ovitrap itself and in combination with larvicides can be used as a control method [36].

There is an issue with measuring the success of vector control actions through traditional vectorial indexes stemming from the differences in the performance of anti-vectorial personnel, the capacity to cover broad areas of houses and identify correctly breeding sites sometimes with high levels of difficulty to be accessed.

In contrast to old-style vector control operations currently, we are facing increasingly large and explosive urbanizations in areas with poor infrastructure, deficient access to water, sanitation (and refuse systems) [11].

The advent of more complex methods of measurement based on statistical modeling requires to assess the relationship between entomological indexes and densities of adult mosquitoes in an area and the risk of transmission and to use the newly acquired computer technology in producing consolidates in real time to feed urgent decision making in vector control and identify areas of failure or success in real time.

10. Community involvement

Mosquito control has been in most countries a responsibility of the governments and the level of engagement from families and communities has been relatively low but recent studies demonstrate that there is great potential for population participation and collaboration in anti-vectorial control with the advantages of overcoming the logistic difficulty of visiting households to perform an effective control.

It is fundamental to the understanding of vector control that it has been traditionally managed in a vertical way taking as a model the Malaria and Yellow fever Campaigns of the beginning of twentieth century with a quasi-military hierarchical structure where all initiatives and directions followed a top-down format [6, 37].

During several decades, this vertical structure was functional without many challenges from the population and the members of the vector control structures but in the twenty-first century, there is a tendency to democratize the society and its organizations and to decentralize the decision process [38, 39].

One of the main challenges during the last quarter of the twentieth century and the first decades of the current one is the lack of cooperation with the procedures of source reduction that involve entering the patios, applying larvicides to water deposits, removing useless breeding sites (from the official perspective) that could have potential use for the household members.

How to deal with decreasing cooperation in the communities? There is a need to involve those same communities in the control of their own homes and communities [38]. The perception of vector control needs to be less of fulfilling an imposed and confused sanitary obligation and more of a clear routine to protect health and life of the family members.

The work of vector control now requires knowing more than just the dengue vector and more about the community dynamic, practices, and culture to design more effective and socially acceptable control.

Recent experiences in Latin America and South/South-East Asia for community-based control have demonstrated that there are possibilities to apply innovation in vector control. Most of them have occurred because of a reassessment of the relationship between communities and their vector control [38].

Ethnographic studies have provided light on how perceptions of discardable, conservancy deposit management relate to cleanliness and hygiene aspirations in the family and their relation to disease, the need for health care/hospitalization, and risk of death [11].

As part of the knowledge required to improve vector control by the family is what are the social roles of the members in a family nucleus. The domains or responsibilities of mother and father need to be understood to be effective in tailoring effective messages to community members.

Depending on the culture, there are gender-based roles in the maintenance and elimination of potential breeding sites and this knowledge will provide a more clear effort to target the individuals in charge of keeping the containers free of mosquito sources [14].

The concept of hygiene and cleanliness needs to be linked in the communication to the preservation of health and the prevention of a spectrum of diseases and health disorders, and in this way, the removal or neutralization of breeding sites becomes relevant to the community as it is now to the vector control worker and the Ministry of Health.

Once vector control ceases to be important only for the vector control worker and the government institutions to some degree, it is important to operationalize a transference of responsibility to the individual, the family, and the community.

There are actions to be taken to transform the vector control of a routine in charge of the vector worker into a global effort that includes periodical cleaning-up campaigns helping neighbors to get rid of potential breeding sites (plastic objects, old metal pots, tires, cans, and similar) being careful not to stimulate the turn-over of old containers to brand new ones. As a personal testimony, many people observe that after a cleaning campaign, there is a tendency to replace those articles taken as refuse with new ones, and new breeding sites will be placed in the patios and backyards.

The second change in the vector control is to modify the profile of the vector control personnel into a more polyvalent profile, providing them a more comprehensive training to become more of an environmental care officer.

11. Community involvement: Schools, neighbor associations, and local governments

In the past, many public health programs were disease-specific and the members of the households were required to participate in programs that cause temporary or low motivation to them. In the case of dengue, the benefit for the household and the community seems to be scarce. Are they only acting for dengue control or does their participation lead to real family and community improvement?

Unless we are facing a dengue epidemic the main concern of the population is the nuisance of mosquitoes biting the house dwellers but they have more urgent needs to solve such as the perennial crisis with water supply, the accumulation of garbage, and the irregular refuse system or the total absence of one. Curiously, these felt needs are related to mosquitoes and dengue. There is a need to get a trade-off with community members to act on mosquito control as part of a more comprehensive package of community improvement measures.

Some communities facing the difficulty of getting rid of trash and other solid waste have organized themselves to pay individuals to mobilize their refuse in their own vehicles when the local governments are not able to do it. There is a real concern for the elimination of trash reinforced with the knowledge of dengue and similar mosquito-borne disease and the presence of their breeding sites in their homes.

Neighbor associations have demonstrated that if they identify a problem such as disease/s caused by mosquitoes, they are highly receptive to orientations leading them to take action, raise awareness in their own neighborhoods about control of trash and adequate control of water deposits, and even advocate for projects to provide better and more frequent water supply and wastewater systems.

In countries such as Honduras and Puerto Rico, there have been joint partnerships of the private and public sectors involved in the control of breeding sites and the School nucleus of teachers, parents, and students, which has been expressed in the production of educational material including textbooks and workbooks.

The development of school modules has followed a long process since the genesis of the idea as a research project in Latin American countries with a component of formative research, with rigorous measurement and the partnership of schools. Initially, the idea was to emphasize the dengue control component but later it was identified the need to incorporate environmental components based on the adequate water supply and the care required by the deposits containing water indoors and outdoors, and the component of adequate disposal of domestic waste. Finally, the last modules center on water deposits and solid waste more relevant to dengue control. More details were provided in the dengue module about concrete actions needed from parents and school-age children. The learning objectives were reached and the next step was the application of skills in the practical tasks of developing the actions of control in the family and with community members [40–42].

The Environmental School Program (PEA, for its Spanish acronym) is a dengue control initiative focused on primary schools that took place during 2005–2010 in several cities in Honduras. The environmental health program was designed to increase knowledge and develop skills in the identification and control of *A. aegypti* breeding sites, as well as in water and solid waste management [41] as mentioned before.

Incorporating through the school, young school children, their parents, and teachers can provide sustainability to a renewed vector control program targeting the

action on dengue transmission but improving the environmental conditions at home and in peri-domestic spaces. Internalizing some values on domestic hygiene seems to be the route to long-term control.

Communities are also mobilized in the development of the activity called D-days when every household assumes the responsibility to clean their water deposit and eliminate discardable containers, and the government institutions provide support for an effective refuse system.

It is important to mention that experiences are crossing borders, and in many countries, the school system and the local government adjust experiences to their local circumstances. Scientific community has a role in supporting the development of innovative methods, diffusing them through scientific literature and institutional communications, and doing an appropriate and intense advocacy for the adoption of new techniques. Only an active scientific community can lead to changes in the routines of control and the assignment of more responsibilities to local authorities (decentralization) and empowerment to the population to take part in the reduction of the *A. aegypti* populations.

12. Last comments: where do we go?

Policy makers need to know that dengue is a recurrent problem for most countries, and the possibility to obtain an acceptable vaccine is still uncertain and the only known and used control method is vector control, which is potentially suitable as part of broader environmental health measures.

Paradoxically, we have as a result of a long tradition of vector control the emergence of a multiplicity of control methods that remain as collection of effective laboratory and field tests waiting to be taken to a national level, upscaling them in a careful but decided adoption.

It is not possible just to use one single method for vector control, but a combination of them according to needs, availability, and access of expertise by the personnel.

From experimental pilot, experiences are important to rescue the opening of different channels of communication with the communities through the vector-control workers, teachers, students, local governments and including the private sectors and grassroots organizations that have a real interest in all processes improving the life of citizens.

The vulnerability to dengue transmission comes in many places especially in poor neighborhoods because of the chronic lack of water supply, which presses the community dwellers to keep their water deposits that are necessary for their daily routines of cleaning/hygiene, laundry and more important for drinking and preparing their food. It is a common experience for vector control workers to witness the despair and anger of neighbors pressed to use larvicides in water deposits that change the appearance and odor of the water, or when forced to empty some positive deposits depriving them of water that is needed for them.

There is a need to link mosquito (*A. aegypti*) control to the development of programs to provide reliable water supply that will turn clean-up campaigns, temephos applications, or containers emptying from real nuisance to acceptable ways to protect family health.

In recent years, several epidemics of dengue and other *A. aegypti*-borne infections have affected the Americas, and South and South-East Asia, with outbreaks as well in the Pacific Islands and Africa. It is time that the national authorities are convinced

that vector-control measures need to be redirected to be a tool for multi-disease control beyond only dengue and severe dengue. This would lead to alliances with other actors to combine control strategies and develop synergic actions that will have less opposition in the population and more strategic allies.

An area that is essential to the success of vector control is communication, and in an increasingly democratic world, the effectiveness of health programs is based on effective communication between institutions (Vector Control/Ministries of Health) and the population. Once many countries are passing from authoritarian regimes to more democratic institutions, the type of health communication needs to change from unidirectional to bilateral and multidirectional providing an opportunity to gather new ideas and needs from the population to optimize the implementation of new ideas for vector-control and community improvement.

Health policies related to dengue reduction and vector controls need to be shared with the national audiences in a clearer way, and to be open to observations, contributions, and dissent if that happens. As we have mentioned in this vector-control overview, there are many community issues that only solved will provide opportunities for a fully successful control.

There is an increasing level of understanding in the World Health Organization of the role of vector control in reducing dengue transmissions, and once a safe vaccine for all is reached, there must be an effort to use both approaches in a synergic approach. Previous discussions about abandoning the efforts of vector control once the vaccine was reached are practically over especially considering that vector control can have a synergic effect with the vaccine.

With the advent of other pandemic diseases like COVID-19 with more media attention, it is a priority to adopt new control strategies, and identify and integrate allies in the expanded approaches to reduce Aedes populations while a higher purpose: improvement of peridomestic spaces with less trash and wiser use of water containers is achieved.

13. Conclusions

Dengue is a viral infection with the main mode of transmission as vector-borne infection. Its clinical range goes from an asymptomatic infection to a severe lethal disease. We are relying on the prevention of dengue in effective control of the *A. aegypti* mosquito. There is a broad range of options for vector control but the most widely used are based on insecticides that are the cause of debate because of their potential environmental toxicity but also by their increasing report of resistance. Recent WHO reports state their use in the different world regions with variable levels of the result.

Most of the effort to control *A. aegypti* population concentrate on the reduction of breeding sites and the population of immature stages, while the use of adulticides is used when there are evidences of active transmission of infection and high densities of the mature adult mosquitoes (outbreaks of disease).

Currently, there are many studies on alternative methods that show high effectivity and efficacy when treating breeding sites, there is an urgency to implement the new methods outside of its research context adopting a more operational process.

Technology resources need to be applied to the challenges produced by unorganized urban growth, limited personnel, passive resistance to authoritarian styles to perform breeding sites assessment and control.

Modern times require new approaches including the adoption of new techniques for control, reviewing the profile of the vector-control worker and the organization in

its entire structure, and a necessary process of relearning how to be more effective in interacting with the communities and the civic organizations.

Populations need to know better what is done in vector control in their own homes to turn a passive and sometimes hostile attitude into a more cooperative one, incentivizing their participation in the control through neighbors and civic organizations and as it is proposed through their school systems: learning and participating in their own domestic hygiene, which includes the vector's breeding site control.

There is a need to develop a more integrated approach with other disease-control programs and privileging a more decentralized process to perform disease control.

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Conflict of interest

The authors declare no conflict of interest.

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Chapter 15

Perspective Chapter: Hospital Disaster Management during Dengue Outbreak

Ashis Shrestha

Abstract

The outbreak of dengue results in surge of patient in the hospital. Dengue without warning signs are usually treated on outpatient basis while those with warning signs presents to emergency and are treated as inpatient basis. Severe dengue is treated in intensive care unit. This creates the challenge in managing the surge from outpatient to intensive care unit, often exceeding the capacity to treat. A hospital needs disaster management plan to cope with this surge of the patient. The disaster plan includes, hospital incident command system, networking plan, surge capacity, and emergency system. Beside this, a dynamic protocol needs to be implemented as sensitivity and specificity of the test kit remains same however, the predictive value of screening question increases as more and more population get affected. Therefore, primary or screening triage plays important during the surge of the patient.

Keywords: command system, dengue outbreak, disaster management, hospital, surge capacity

1. Introduction

Disasters are serious disruptions to the functioning of a community that exceeds its capacity to cope using its own resources [1]. Similarly United Nations International Strategy for Disaster Reduction (UNISDR) defined disaster as "a serious disruption of the functioning of a community or a society involving widespread human, material, economic, or environmental losses and impacts, which exceeds the ability of the affected community or society to cope using its own resources" [2]. A similar definition is used by World Health Organization. These are universally accepted definitions, and it holds true for a country, community, and hospital as well. Hospital's ability of cope using its resources is affected when the number of patient flowing in the emergency exceeds its capacity.

Disasters are caused by hazards which is "a process, phenomenon or human activity that may cause loss of life, injury or other health impacts, property damage, social, and economic disruption or environmental degradation" [3]. There are various type of hazards like environmental, technological, biological, etc. The risk of disaster is directly proportional to hazard and vulnerability. The vulnerability is "the condition determined by physical, social, economic and environmental factors or processed

which increase the susceptibility of an individual, a community, assets or system to the impacts of hazards" [4]. Hazard is not preventable; however, disaster can be prevented by managing vulnerability, known as risk management. The vulnerability of the hospital increases with poor hospital structure, uncoordinated patient flow and crowd control, absence of triage, inappropriate emergency management, and poor record-keeping system on normal days. Disaster is an escalation of normal day emergency; therefore, failing to manage daily emergency is failing to manage disaster.

There are four phases in disaster cycle, preparedness, prevention or mitigation, response, and recovery, **Figure 1** [5].

During the outbreak of dengue, the country, community, or a hospital responds to the event. The effort that is collectively put together in the response phase improves the response to some extent but not as desired. Therefore, for a good response, effort must be invested in the preparedness and prevention phases of disaster cycle. Similarly, recovery is an important phase after response.

The global burden of dengue has been rising in the last 30 years due to urbanization, climate change, and increased mobility. The rise is more in South-East Asia and South Asia [6]. A systematic review considering 262 outbreaks observed between 1990 and 2015 had 112 outbreaks after 2010, and the total number of patients since 1990 was 291,964 [7]. The patient with dengue presents with fever and myalgia and may require hospital admission for intravenous fluid. Out of patients visiting the hospital, nearly 40% require intravenous fluid [8]. This means an increase in the influx of patients visiting hospital and the number of admissions compared to normal days. This will cause a shortage of hospital beds, a shortage of medical supplies, and a risk of loss of revenue from cancelation of elective procedures. Moreover, the risk of nosocomial dengue has also been reported in health care workers [9, 10]. This surge will also cause exhaustion of health care worker. In a cross-sectional study, high burnout was observed in 15.9% of health care workers [11].

It is evident that the outbreak of dengue has been increasing in the last 30 years, causing a surge of patient in health care facilities overwhelming the service and

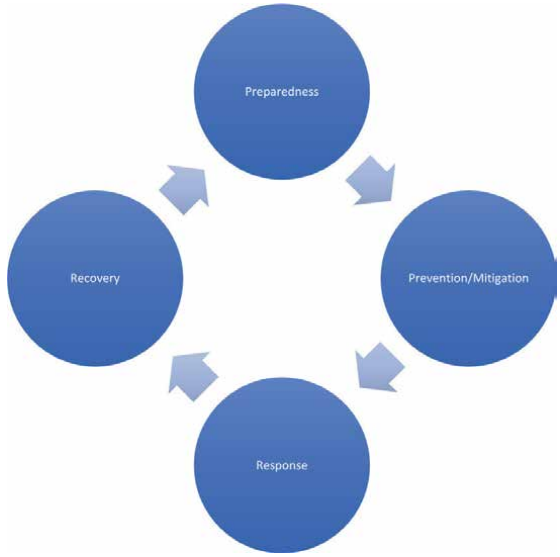

Figure 1.
Disaster cycle.

human resources. This is a hazard leading to a challenge for space management for the patients' surge. Furthermore, in such conditions, it takes a lot of effort to maintain the supplies of fluid, medicine and blood products and ensure adequate Intensive Care Unit (ICU) beds are available [12]. Patient safety is compromised due to the pressurized health care system of the hospital. Therefore, risk management requires improving the response, which further requires improving preparedness. Planning the response, developing strategies, tactics, and implementation planning is important to improve the response.

2. Hospital disaster preparedness and response plan

Sendai framework for disaster risk reduction (2015–2030) priority 4 emphasizes enhancing disaster preparedness for effective response [13]. Therefore, a hospital requires multi-hazard hospital disaster preparedness and response plan. This plan will be useful in any type of disaster, including the outbreak of dengue infection. The outbreak of dengue will result in a shortage of space, unavailability of beds in critical care units, increased workload of the staff, and decreased quality of care for the patients [14]. This needs to be addressed in disaster management plan. Important components of hospital disaster management plan are as follows.

2.1 Hospital incident command system

The outbreak of dengue will last for several weeks; at the same time, the hospital also needs to manage regular daily patients. The hospital incident command system is the pillar of disaster management in the hospital [15]. Activation of hospital incident command. **Figure 2** will help in prioritizing and executing the task. The role and responsibilities of the individual are designated. The incident command is controlled by incident commander, who is the chief of the hospital. Planning for the management of the dengue and regular patient is done by planning officer. This includes communicating, coordinating, and managing staffs and space. The operational officer will coordinate clinical management and protocols. The logistic officer is responsible for maintaining supply–demand chain, while finance officer is responsible for financial planning.

The disaster management plan cannot cover all aspects of dengue management from the first to the last day. To manage the outbreak in a daily basis, incident action plan is prepared (IAP), **Figure 3** [16]. The action starts with incident notification and initial response, followed by incident command meeting. The incident command meeting prepares IAP, which is executed after operational briefing; this is called operational period. Once the operational period starts, preparation for next operation

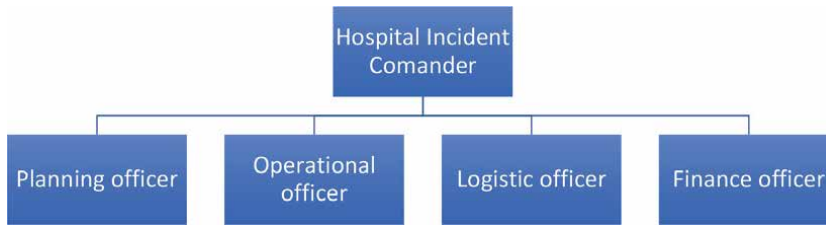

Figure 2.
Hospital incident command system.

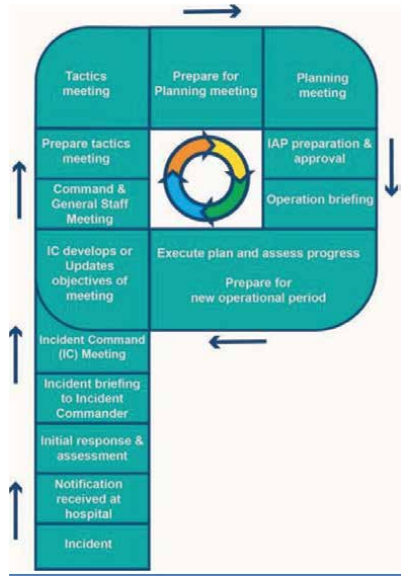

Figure 3.
P diagram of Incident Action Plan.

period is done considering the situation, information, and lesson learned during the preceding operational period.

2.2 Networking plan: communication and coordination

Networking plan is an important component of surge management in dengue. There are two types of communication that needs to be planned in preparedness phase. External and internal communication: the external communication includes the communication with the local authorities of the country and local stakeholders. This communication is necessary for case reporting and advocacy of the preventive measure. The prevention and control of the outbreak is an effective measure for decreasing the surge of the patient in the hospital. Moreover, during the surge of patient as the space of hospital is overwhelmed, other hospitals in the region of outbreak, which do not have full bed occupancy, can be used. This coordination needs prior planning. One of the models tested during COVID-19 is the hub and satellite model [17]. Hospitals in the country are divided into hub according to the region and its resources; the hospitals that are near hub hospitals are categorized as satellite hospitals. A communication and coordination protocol for hub and satellite hospitals to support each other during the disaster is established during the preparedness phase [18]. Internal communication requires a communication officer with lists of contacts of all hospital staffs. Staffs needs to be informed about the communicable potential of dengue, the precaution to be taken, and the protocols of clinical case management and surge management needs to be informed to all hospital staffs.

2.3 Surge capacity

During the outbreak of dengue, most patients visiting outpatient department can be sent home, and some patients visit emergency and require fluid management along with

admission. In an observational study, outpatient treatment was needed in 82% of patient, and 18% required hospital admission. Intravenous fluid resuscitation was required in 3.2% of the patient [19]. A systemic review published in 2019 summarizes that the countries' dengue endemic has seasonal surges in the hospital; they have early warning system, therefor they cope with the surge by temporary expansion of the surge. The expansion includes, space, human resources, laboratory services, funds, and logistics. However, in non-endemic countries, surge are managed by reverse triaging. Therefore, context-specific planning will help hospitals cope with the surge of patient during dengue epidemics [12]. All hospitals face challenges in their ability to meet the surge demand. Larger hospitals in regional settings usually operate near capacity, while smaller hospitals at local level operate with limited availability of resources. Therefore, coping with surge is a challenge for all hospitals and needs to be planned during planning phase of disaster cycle [20].

Key components of surge planning include four S's: Structures, Staff, Stuff, and System [21]. Sub-acute units in the hospitals should be identified as it is much easier to manage the outbreak if it can be managed inside the facility. However, if the facility is unable to meet the demand of surge the spaces outside the facility need to be considered [22]. Expansion of the space requires effective management of staffs and stuffs. There should be interdepartmental coordination to manage the staffs from the department that does not have a patient load; moreover, hub and satellite [18, 23] mechanism is also helpful to mobilize staffs from the hospitals that are not expecting surge of patients. Moreover, equipment required to expand the capacity, needs to be planned. The equipment may include ward bed, ICU bed, medications, and logistic supplies [21]. An effective multi-hazard management plan must describe the system with a clear guiding policy in case of surge of patients.

2.4 Emergency system

Emergency system of the hospital needs to be functional on a daily basis because disaster is an escalation of everyday work. During disaster, it is not possible to implement a new system that has never been in practice. Therefore, hospital requires planning of outpatient services and emergency services. Management of the patient at triage, emergency and inpatient ward, and ICU must be well coordinated. The essential component of efficient management is a functional triage system. A primary and secondary triage is required to sort out and prioritize patients. Primary triaging is a system based on questionnaire. During the outbreak of dengue, the primary triage may contain questions like, "Do you have fever?" If the answer is yes, then the patient goes to dengue suspect zone, however, if the answer is no, then the patient goes to regular outpatient or the emergency. Once the patient is sent to the dengue suspect zone, secondary triaging is done, where patients are triaged based on symptoms and signs and labeled as critical or non-critical [24]. During the outbreak of dengue, the surge of the patient might interrupt the services for the patient with other clinical conditions presenting in outpatients, emergencies, and inpatient. Therefore, a separate unit consisting of dengue emergency, outpatient, inpatient, and critical care patient needs to be planned [25]. Even countries with infectious disease hospitals may be overwhelmed with the surge of the patient causing overflow of the patient to other hospitals.

3. Protocols

Protocols are important for the consistency of the service delivery. There is WHO management available for dengue management [26]; moreover, every

dengue-endemic country will have national guidelines. These guidelines are more static and are based on recently available evidence. Every healthcare institution needs to have a protocol based on these guidelines. These are operational, brief, and dynamic documents. During the dengue outbreak, the protocols are adjusted based on the clinical evidence for patient safety and surge of the patient. Therefore protocol needs to be dynamic and needs to be changed according to the situation [27].

Fever is the most common presentation of dengue followed by myalgia [19, 28]. For all patients with fever, the screening is done by rapid diagnostic kits that detect NS1 antigen and IgM antibodies. The NS1 antigen is detectable the most within the first 2–4 days, and IgM antibody after that. During this period, the sensitivity of the kit to detect NS1 ranges from 63% to 73%, while that of NS1 and IgM combine ranges from 90% to 98% [29–32]. The important phenomena, is that the sensitivity and specificity of the test kit remains same however, the predictive value of screening question increases as more and more population get affected [33]. As laboratories are overwhelmed with the surge of the investigation, it becomes very difficult to process the test in time, affecting sick patients whose clinical diagnosis is in a dilemma. Therefore, at the peak of outbreak, we may not need to send investigations for all patients who do not have warning signs. Hence, a small change in protocol for investigation or management will cause huge relief of workload to the hospital during an outbreak.

Dengue virus can present with severe cases and mortality in 1–5% of cases. The important laboratory finding of dengue fever is thrombocytopenia [34]. Platelet counts are useful in predictive and recovery parameters of dengue fever, dengue hemorrhagic fever, and dengue shock syndrome [35]. Studies suggest a high risk of bleeding below a platelet count of 20,000/cumm and a moderate risk below 21–40,000/cumm [36]. The cut-off value of less than 46,500/cumm has also been taken to stratify the risk of bleeding [37]. Platelets transfusion is found to be done in some literature [19], however, it has not been proven to be effective in preventing the development of severe bleeding or shortening the time to the cessation of bleeding [38]. Therefore, this type of information needs to be analyzed carefully and must be on the basis of the best available evidence before applying it to protocol. During the outbreak of dengue, the protocol must be customized so that it does good to maximum number of patients.

4. Capacity building in clinical case management

It is necessary that health care workers have a good knowledge of dengue infection. A health care worker in a hospital must know the screening criteria, treatment criteria, reporting procedures, and preventive knowledge. The knowledge does not always translate to the adoption of preventive measures [39].

Training on infection prevention and control are necessary while orientation on protocol is important to ensure that all staffs have uniformity in action. Moreover, during the outbreak of dengue, individual roles and responsibilities must be understood by each staff. This can be achieved by simulation exercises. There are various types of exercises that can be planned according to preparedness of the hospital [40]. Tabletop exercise can be done to test the dengue outbreak management plan. The plan is adjusted and finalized according to the lesson learned from the tabletop exercise. After finalization of the plan, functional components of the plan like primary and secondary triage can be tested by drill. Similarly, drill can also be done with clinical

case management and reporting system. The coordination and communication can be tested with functional exercise. Finally, full scale simulation exercise can be planned during the preparedness phase.

Besides this online learning has been one of the effective ways of learning following the COVID-19 crisis. Online learning is no more an option, but it is a necessity [41]. During the surge of dengue patient, the event will exhaust human resources. It will be difficult to manage health care workers' time for educational sessions. This method of learning can be adopted during time of crisis. However, inventing new methods during the crisis is not helpful; therefore, online sessions must be part of professional development for health care workers.

Social media is yet another powerful way of disseminating information. This platform can be used for short messages or updates. During disaster, there are three types of social media users: influential social media creators, followers, and social media inactive [42]. Social media is used as reporting system, distributed problem-solving, and digital volunteerism [43].

5. Database system

The surge of dengue outbreaks can be managed effectively by maintaining proper database system in the hospital. The real-time data will help in planning human resources and logistics. Moreover, predictive analysis can be done to anticipate the actions that need to be taken in future days. There are some important variables that will provide crucial information for patient management. For example, the number of cases per day will identify the trend of the dengue, and the address of the patient presenting will help identify the outbreak area. This will further help in the control of the disease. Likewise, the presenting symptom of the patient is helpful in understanding the pattern of presentation. This is important during the surge of the patient when the diagnostic facility is overwhelmed, and decisions have to be made on the basis of clinical findings for non-severe cases.

Data without analysis is not useful; therefore, a system of analyzing and providing the information to the concern in a useful and understandable way is important. This system must be in place prior to the crisis. The activities that are habitual and are in

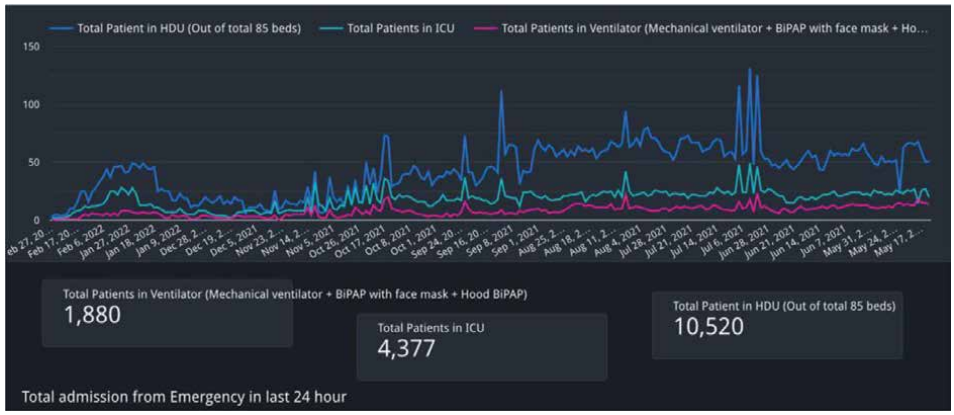

Figure 4.
Sample of dashboard used during COVID-19 pandemic.

daily practice work well during the crisis. One way of working is by using a dashboard that is visible to all clinicians, **Figure 4**. Moreover, hospitals should also have the capacity to collect data from external sources, which means the events that are being reported from other hospitals, recent advances, and updates. The process of acquisition of internal and external information, and its analysis to produce a meaningful information is an essential component of disaster management [44].

Research is another integral part of disaster management. Researches can be planned in two phases: during preparedness and response phases. In countries where dengue is endemic, response researches can be pre-planned. This will help prepare for subsequent outbreaks and improve the response.

6. Conclusions

Dengue is an infectious disease that can potentially exceed the hospital's capacity to provide the service. This surge of the patient can be managed by disaster management plan. Every hospital must have a disaster management plan including the hospital's outbreak management components. The four phases of disaster cycle need to be addressed well. An investment of effort in preparedness will improve the response phase of the disaster.

Conflict of interest

None.

Notes/thanks/other declarations

None.

Acronyms and abbreviations

COVID-19	Coronavirus disease 2019
ICU	Intensive Care Unit
IAP	Incident Action Plan
WHO	World Health Organization

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Dengue Fever in a One Health Perspective - Latest Research and Recent Advances presents studies on dengue fever (DF) and dengue virus (DENV) that discuss eco-epidemiology, physiopathology, and new biotechnological tools to fight against this important disease in the context of the World Health Organization's One Health strategy. The book is organized into five sections: "Epidemiological Aspects", "Environmental Aspects", "Pathogenicity", "Diagnosis and Treatment" and "Management Strategies". The chapters address topics such as DF prevalence and management in a Chinese county, the risk of DF in American children younger than 15 years, the silent transmission of DENV by asymptomatic individuals, the use of X-ray and ultrasound to identify severe DF cases, gene-silencing techniques to investigate biological aspects of DF, viral genomic surveillance to promote early intervention in DF epidemics, and much more.

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