An Overview on the Prevalence and Diagnosis of Dengue

Submitted by- Ashik-Uz-Zaman

ID: 11126005

September 2018

Microbiology Program

Department of Mathematics and Natural Sciences

BRAC University

Bangladesh

An Overview on the Prevalence and Diagnosis of Dengue

Name: Ashik – Uz – Zaman
ID: 11126005
Department of Mathematics and Natural Sciences
Program: Microbiology

Declaration

I hereby declare that the review paper „‟An Overview on the Prevalence and Diagnosis of Dengue'', has been written and submitted by me, Ashik-Uz-Zaman and has been carried out under the supervision of Dr M. Mahboob Hossain, Professor, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University, Dhaka.

It is further declared that this review paper has been composed solely by me and it has not been submitted, in whole or in part, in any previous institution for a degree or diploma. All explanations that have been adopted literally or analogously are marked as such.

_____

(Ashik-Uz-Zaman)
Candidate

_____

Certified by
(Dr M. Mahboob Hossain)
Supervisor
Professor Microbiology Program
Department of Mathematics and Natural Sciences
BRAC University, Dhaka

Acknowledgment

I acknowledge my esteem to Professor A F M Yusuf Haider, Chairperson of MNS Department of BRAC University for allowing me and encouraging me to complete my undergraduate review paper. I am much beholden to Professor Shah M. Faruque, Coordinator of Microbiology Program of Department of Mathematics and Natural Sciences, BRAC University

My regards, gratitude, indebtedness, and appreciation goes to my respected Supervisor Professor Dr. Mahboob Hossain for his constant supervision, constructive criticism, expert guidance, enthusiastic encouragement to pursue new ideas and never-ending inspiration throughout the entire period of my research work. I would like to thank and express my most profound gratitude for guiding me in my report writing and providing time to time suggestions regarding the work.

I would also like to thank my parents, my sister Sarah Mehzabin. They have always supported through thick and thin. And I will still be indebted to them.

I would love to take this opportunity to mention the names of some of my closest people who really helped me a lot in this life-time and inspired me to stay stuck with my work. Shahnewaz Hossain Sabbir, Mahadi Ezaz Maswood, Mahmud – Un – Nabi, Mushfiq Shihab, Akash Ahmed, Salman Al Azmir Simon and Digonto Islam, thank you so much!

Ashik-Uz-Zaman

September 2018

Abstract

Dengue viral contamination has become greater in size, global health concern with over two-fifths of the world's population at risk of infection. This paper provides a brief review of the history of dengue research. It is the most rapidly laying out measure borne disease, attributed to changing demographics, urbanization, environment, and global travel. It continues to be an intimidating remark in over 100 tropical and sub-tropical countries, affecting predominantly children. Dengue also carries a hefty financial burden on the health care systems in affected areas, as those infected seek care for their symptoms. The search for a suitable vaccine for dengue has been ongoing for the last sixty years, yet any effective treatment or vaccine remains elusive. A vaccine must be safeguarded for all four serotypes of dengue and be cost-effective. Many proceed towards to developing, candidate vaccines have been employed. The candidates incorporate live weakened tetravalent vaccines, chimeric tetravalent vaccines based on attenuated dengue virus or Yellow Fever 17D, and recombinant DNA vaccines based on flavivirus and non-flavivirus vectors. Dengue viruses spread the disease to nearly 100 million human beings each year living in 110 countries spread over all tropical areas on earth. Tens of millions of dengue illnesses occur annually including the hundreds of thousands of children who are hospitalized for dengue hemorrhagic fever. A health problem of this scope should be regarded as a high priority and should have attracted ample funding from donors and national authorities. But such is not the case. A brief historical review reveals that there was a greater number of laboratories and a greater allocation of resources to dengue research 30-50 years ago than there is today. WHO needs to provide leadership in promoting dengue research. Each and every dengue-endemic country should realize the fact that a sustained research capability is crucial to resolve the long-term ongoing problem of dengue control(1).

Introduction

Dengue is an acute feverish disease caused by the mosquito-borne dengue viruses, consisting of four serotypes, that are members of the flaviviridae family, genus flavivirus. All four DENV serotypes have emerged from syllabic strains in the forests of South-East Asia. DENV is presently the most common cause of arboviral disease globally, and all four serotypes of DENV can be found throughout the world(2). More than 100 countries are endemic in a state, primarily affecting 2.5 billion inhabitants in the tropical and subtropical regions as well as 120 million travelers to these regions every year. The World Health Organization estimates an annual incidence of approximately 100 million infections, with approximately 500,000 people with dengue hemorrhagic fever (DI-IF) requiring hospitalization, a large proportion being children. DHF may develop into dengue shock syndrome (DSS) whereof the mortality rate is approximately 1-2.5% in ratio(3). Successful treatment of patients with DHF and DSS is labor intensive and expensive, but without proper treatment, fatality rates may exceed 20%.

World Perspective of Dengue

Viruses are tiny representatives that can infect a variety of living organisms, including bacteria, plants, and animals. Like other viruses, the dengue virus is a microscopic structure that can only replicate inside a host organism for its growth and survival(4).

Discovery of the Dengue Viruses:

The dengue viruses are members of the category Flavivirus in the family Flaviviridae. Along with the dengue virus, this genus also includes a number of other viruses transmitted by mosquitoes and ticks that are responsible for human diseases. Flavivirus mainly includes yellow fever, West Nile, tick-borne encephalitis viruses and Japanese encephalitis(5).

In 1943, Ren Kimura and Susumu Hotta first isolated the dengue virus. These two scientists were studying blood samples of patients taken during the 1943 dengue epidemic in Nagasaki, Japan. A year later, Albert B. Sabin and Walter Schlesinger independently got involved in remoting the dengue virus(6). Both groups of scientists had isolated the virus now referred to as dengue virus 1 (DEN-1).

Global status of dengue and dengue hemorrhagic fever:

Dengue viruses spread about in nature as four antigenic ally-related serotypes, the only such group among the arthropod-borne viruses. Each of the four serotypes has progressed into multiple genotypes(7). The viruses are perpetuated in nature in two cycles, a jungle cycle in which several syllabic mosquito species mediate viruses to several species of sub-human primates, and an urban cycle in which the virus is transmitted predominantly by *Aedes aegypti* to human beings. The dengue viruses are unique in that a single dengue infection may

"sensitize" individuals to severe and fatal disease accompanying infection with a second serotype(8).

In response to a number of 20th century phenomena, the distribution and population of *Aedes aegypti* and the global burden of dengue have grown dramatically in recent decades(9). With increase exponentially human, populations, urbanization and the development of rapid transport systems, dengue fever and dengue hemorrhagic fever/dengue shock syndrome, now occur in over 100 countries and territories (10). They cause an estimated 50-100 million infections among the more than 2.5 billion people at risk in urban, peri-urban and rural areas of the tropics and subtropics. While the full burden of dengue infections is not known, each year it is estimated that some tens of millions of persons experience classical dengue fever and another 500,000, mainly children, are hospitalized for DHF/DSS(11). Death rates are as high as 5% in some areas. Dengue is now endemic in the American, Western Pacific and South-East Asian regions of WHO. Few parts of the African and Eastern Mediterranean regions are affected as well.[i] Prior to 1970, only nine countries in the world had experienced DHF/DSS epidemics; by 1995, the number had increased more than four-fold. In the 1950s, an average of 908 DHF/DSS cases were reported each year(12). For the period 1990-1998, this average had increased to 514.139 cases. In 1998, a total of 1.2 million cases of dengue and DHF were reported to WHO, including 3442 deaths.

Global status of dengue research and control:

Dengue research started early in the 20th century before the isolation of virus(13). The clinical and laboratory features of dengue, the viral status of the involved agent, the susceptibility of monkeys and the vector status of *Aedes aegypti* were established in a series of well-designed human volunteer studies. Dengue viruses, types 1 and 2, were isolated in suckling mice and characterized during the time of second world war(14).

Research milestones: In the late 1950s, the clinical syndrome, DHF/DSS, was described and attributed to dengue infection(15). A decade later, it was recognized that DHF/DSS accompanied second dengue infections and, unique in human medicine, during initial dengue infections in infants who were born to dengue-immune mothers. Subsequent epidemiological studies, a monkey model and numerous in vitro observations provided an explanatory mechanism, antibody-dependent enhancement of dengue infection(16). This is based upon evidence that dengue viruses replicate in cells of mononuclear lineage in human beings(17). These Fc-receptor-bearing cells are efficiently infected following attachment of complexes of dengue virus and non-neutralizing IgG antibodies. From the 1970s, with the emergence of modern immunology, the role of cellular and hum oral immunity, the molecular mechanisms of inflammation and the control of dengue infections were studied(18). During and subsequent to the 1980s, full-length sequences of the dengue genome have been described for multiple strains of all dengue serotypes.

Facilities: During the 1950s dengue studies were performed in field laboratories maintained by the Rockefeller Foundation in Trinidad, Brazil, Africa and India, and by colonial research institutes located in South and south-east Asia(19). A decade later many of these research networks were phased out and replaced by national public health laboratories, a network of U.S. military infectious diseases research laboratories (Thailand, Malaysia, Philippines, Indonesia, Peru, Brazil and for a brief time, WHO vector research units in Thailand, Indonesia and India. Today, dengue research is supported by intramural funds in government research institutes of the larger developed countries. The biggest cadre of scientists is in the U.S. public health service and military research laboratories. As compared with chronic diseases, dengue is regarded as a low-priority health problem for the developed countries. A minimum amount of support for dengue research is offered and served to a small number of university scientists in some developed and affluent developing countries(20).

Control Programmes: During the 1940s, a historically unique mosquito control programme was initiated(21). By 1960, under the leadership of the Pan American Health Organization,

Aedes aegypti had been eradicated from most major South and Central American countries(22). After this achievement, however, many of these programmed were dismantled and within two decades, the vector regained its former range.

Basic virology: Progress in basic virology is moving very quickly. The flavivirus field is diverse, small and dynamic(23). Compared with other genera, disease-producing flaviviruses are little used in the study of basic virology.

Vaccine development: Current dengue vaccines under development include: two sets of tetravalent live attenuated viruses (LAV) , a genetically-altered LAV (NII-1), a dengue-dengue chimera (CDC/Mahidol), a yellow fever-dengue chimera (Acambis), an alphavirus replicon (USAMRIID), two naked DNA vaccines (Naval Medical Research Center/USAMRIID), a formal in-Inactivated tetravalent vaccine (WRAIR) and numerous sub-unit vaccines prepared by commercial, university or government laboratories(24). Some of these vaccines are or have been tested in human volunteers(25).

Control methods: Many methods are available to reduce or destroy populations of *Aedes aegypti*(26). Nonetheless, since the days of Dr Fred Soper, no-one has found the right blend of procedures that matches the resources available or is compatible with today's legal systems.

WHO role: The entire field of mosquito control needs to be energized, even re-born.

Leadership and funding are essential.

Control – human behaviour: *Aedes aegypti* breeding sites are largely man-made(27); humans provide the blood that promotes the survival of both mosquito and virus. The activities that promote mosquito breeding are not usually linked to emotion-laden customs or behaviors.

Bangladesh Perspective Dengue

Dengue is a mosquito-borne flushed viral illness. Since its first recognition during the last quarter of the eighteenth century, the periodic outbreak has been reported from both developed and developing countries with Asia always remaining the area of highest endemnicity(28). Until the middle of 20th century dengue was regarded as self-limited, non-fatal febrile illness with occasional hemorrhagic manifestations that only rarely resulted in the severe or fatal outcome. Epidemic dengue with severe hemorrhagic manifestations was first reported in the Philippines in 1956(29), Since then epidemics which came to be known as dengue hemorrhagic fever have occurred periodically in other Southeast Asian regions and term dengue shock syndrome was subsequently coined to describe cases of dengue associated

with increased vascular permeability leading to conditions like intravascular hypovolemia and shock.

Dacca Fever" Bangladeshi perspective:

Although the disease has not been reported in detail from this country, serologic studies and virus isolation conducted on 25 cases during the outbreak of a febrile illness popularly known as "Dacca Fever" that occurred during the summer of 1964 revealed that the condition was due to dengue viral infection(30). The associated severe hemorrhagic manifestations took a heavy toll during the outbreak. Another epidemic fever with features closely mimicking that of DHF occurred again in 1968 in some Bangladeshi areas bordering Myanmar(31).

Since that time sporadic cases and small outbreaks clinically suggestive of Dengue and DHF were seen from time to time by 5 clinicians of the country but these cases remained unreported although entomologic and serologic study reports indicate that the mosquito vector and the entomologic virus both existed during this period in Bangladesh.

An outbreak of an acute febrile illness that started in summer 1999 is currently spreading rapidly in and around the Dhaka city. The illness, occurring in all age groups of both sexes, is often associated with hemorrhagic manifestations and other features suggestive of DHF/DSS and a positive serologic evidence of the condition in the majority of the cases(32).

What exactly is Dengue?

Dengue is a febrile viral illness common throughout the tropical regions of the world(33). In humans, dengue infection causes a spectrum of illness ranging from relatively mild, nonspecific viral syndrome known as dengue fever to severe hemorrhagic disease and death(34). The severe hemorrhagic form of the disease is called dengue hemorrhagic fever (DHF) and dengue shock syndrome (35).

What are the different varieties of Dengue virus?

This virus has four serotypes- DEN-1, DEN-2, DEN-3 and DEN-4. Infection with one serotype does not offer protection against other stereotypes; in fact, a second dengue infection leads to an even worse infection. This manifests as either DHF or DSS which can be fatal. All four serotypes can cause DHF/DSS, but severe disease appears to be more commonly associated with the types known as DEN-2 and DEN-3(36).

What are the vectors for Dengue?

Dengue viruses are transmitted in nature by day-biting Aedes mosquitoes. The most important mosquito vector is the highly domesticated urban species, *Aedes aegypti*(37).

How is it transmitted?

Dengue viruses are not contagious and person-to-person transmission does not occur(38). Transmission requires an infective mosquito (with infected salivary glands) to probe an individual in search of a blood meal. Multiple feedings or probing by an infective mosquito may result in transmission to multiple persons in the same household or building, all having onset of illness within a few days of each other.

Mosquitoes may become infected when they take a blood meal from a viremic person. Viremia'is present for about 24 hours prior to onset and for an average of 5 days after onset of illness, usually coinciding with the period of fever. The infected mosquitoes require a period of incubation the extrinsic incubation period of about 8 to 12 days before they can transmit dengue viruses to another person. During this time the virus grows within the midgut and infects a number of tissues in the mosquito, including the salivary glands.

How long is the incubation period?

The average incubation period is 4 to 6 days but this can be as short as 3 days and as long as 14 days(39).

How long patient remain infectious to others after onset of symptoms?

An infected individual is never infectious to other persons, but remains infectious for mosquitoes for an average of about 6 days.

Can a survivor transmit Dengue to others after she or he has fully recovered?

No. Recovery is complete and there is no relapse or recrudescence of disease.

What is the natural reservoir for Dengue?

The most important reservoir hosts for dengue viruses are Aides mosquitoes and humans.

All four virus stereotypes are maintained in an *Aedes aegypti* - human - *Aedes aegypti* cycle in most urban centers of the tropics, where no other reservoir hosts are present or needed. It is this pool of viruses that played an important role in the current global resurgence of epidemic

dengue in recent years, primarily by airplane travelers.

What has caused Dengue epidemics to end in the past?

Emergency mosquito control measures have been used but these are generally not very effective and most epidemics end because of increased herd immunity. The end of an epidemic does not necessarily mean that the virus has disappeared from the area. During interepidemic periods, dengue viruses are maintained endemically in a mosquito-human--mosquito cycle in most large urban centers of the tropics.

What are the conditions that would lead to an increased epidemic activity?

Aedes mosquitoes like to breed, especially after rains, in standing water such as may be found in flower pots, cans, water jars, artificial ponds, non-biodegradable plastic or cellophane bags and used automobile tires that are discarded in the environment. These are quite common in tropical urban areas, even around hotels.

The factors that have been implicated in the current increase of dengue include, urbanization, overpopulation, crowding, poverty, and a weakened public health infrastructure. Increased international air travel provides the ideal mechanism for the rapid movement of dengue viruses including new strains between populations(40).

Where exactly in the body the dengue virus replicates?

The primary site of dengue virus replication appears to be cells of mononuclear phagocyte but infection of the megakaryocytic in the bone marrow has also been proposed. The Virus's produce a viremia and virus can be isolated from the blood during the acute phase of illness(41).

In DHF/DSS cases, there is generally liver involvement(42). In fulminate hemorrhagic disease, which is not as common, viral particle or antigen have been detected in monocytes in most of the major organ systems including liver, lung, kidney, spleen, lymph nodes and heart, There is, some evidence that dengue viruses can infect the central nervous system(43).

How do I avoid catching it?

The best way to avoid dengue infection is to avoid *Aedes aegypti* mosquito bites.

The mosquito vectors of dengue are day-biters, with peak feeding activity in the morning for several hours after daybreak and in the late afternoon for several hours before dark(44). However, these mosquitoes may feed throughout the day in the shade, indoors, or on overcast days.

To avoid mosquito bites, persons should be aware of the above feeding behavior and use

repellents containing less than 30% DEET on exposed skin at appropriate times and places(45). DEET, especially in high concentrations, can cause serious side effects, particularly in children; therefore, precautions should be taken. Mosquito coils are also useful repellents(46).

The dengue fever typically begins with sudden onset of high fever, severe headache-mostly frontal or retro orbital weakness, malaise, depression, skin rash, and backache, deep muscle, bone & joint pains(47). The disease is known as 'break bone fever' for these last symptoms. Taste aberrations, anorexia, nausea, vomiting and abdominal pain are other presenting features(48). The rash usually appears 3-4 days after the initiation of exposure of the symptoms as diffuse flushing, mottling or pinpoint eruptions that begins on the trunk, spreading out to the face, arms and legs. Conjunctivitis may also be present.

There is often a relative or paradoxical bradycardia in the face of increased temperature. Lymphadenopathy and hepatomegaly may occur but splenomegaly is infrequent(49). Fever and associated symptoms may subside after 3 to 4 days and the patient may recover completely.

Alternatively, the decline in the fever may be followed 1 to 3 days later by a resurgence of fever and symptoms giving a "saddleback" appearance to the temperature curve.

A second rash may appear with the initial decline of the fever. Severe itching, especially of the hands and feet, may accompany this rash, which is sometimes followed by desquamation. The symptoms persist for 1 to 3 days more and then subside with the fever. Mild hemorrhagic manifestations, such as epistaxis, petechiae, gingival bleeding and menorrhagia, are accepted as part of the clinical picture of classic dengue.[ii] Most cases of dengue are benign, ending after about 7 days(50).

How can the DHF/DSS clinically be suspected?

Patients who develop DHF/DSS generally have an onset of illness similar to that seen in "classical" dengue fever.

The critical stage of DHF/DSS occurs as the fever begins to drop around Day 3 to 5 of the illness.

Usually from 24 hours before to 24 hours after the temperature fall to or below normal,

hemorrhagic manifestations may occur, and more importantly, circulatory instability may develop with signs of decreased peripheral perfusion leading to shock. The liver may become enlarged and pleural effusions may develop, usually beginning on the right side.

Disseminated intravascular coagulation and severe gastrointestinal hemorrhage and hematuria may also occur. Manifestations of severe dengue (DHF/DSS) include severe hemorrhage leading to shock through blood loss, sudden increased vascular permeability acute effusion in serous cavities leading to shock with or without hemorrhage and severe hepatitis with encephalopathy(51). Encephalitis with convulsions and/or coma has recently been described with dengue infection.

How is it detected in the laboratory? Diagnosis of dengue infection is best accomplished by obtaining an acute serum sample within 5 days after the onset of illness for virus isolation and antibody testing and a convalescent serum sample 14 to 21 days after illness onset for detecting a in rising IgG antibody titer and/or the presence of antidengue IgM(52).

Most serologic screening for dengue infection is now done with an IgM ELISA(53). With appropriately timed samples, the sensitivity and specificity of this test in diagnosing dengue infection appear to be high. For single serum samples high (40 units or higher) anti-dengue IgM or for a paired sample at least a double rise in ant dengue IgM (from below 15 units to more than 30 units) is considered as evidence of recent acute dengue infection. A high IgG > 100 units with low IgM (< 40 units) indicates recent secondary dengue infection. The IgM : IgG ratios as determined by ELISA may help in distinguishing primary from secondary infections. IgM / IgG ratios of 1.8: 1 or more is considered to be indicative of primary dengue infection(54).

Specific diagnosis of dengue infection is made by isolating the virus from the patient's blood. Virus isolation can be made by mosquito inoculation, and by using C6/36 mosquito cell cultures(55). The virus is detected and identified by immunofluorescence using serotype specific monoclonal antibodies. The virus may also be detected from serum by amplifying dengue viral RNA by PCR technology.

What is the treatment of DHF/DSS?

Suspected DHF/DSS cases should preferably be hospitalized, since shock may develop in about one-third of the patients(56). Patients should be carefully watched for any signs of clinical deterioration or warning signs of shock which usually occur on or after the third day of illness. The rather constant finding that a decrease in platelet count usually precedes the

rise in hematocrit is of great diagnostic and prognostic value. In order to be able to recognize the evidence of a capillary leak syndrome & early signs of shock and thus take preventive action, platelet count and hematocrit value should be estimated daily.
Patients with mild DHF can usually be rehydrated orally and an antipyretic drug may be all that is needed. Salicylates should be avoided(57).

Patients should be treated immediately by intravenous fluids if there are any signs or symptoms of shock, a sudden rise in hematocrit or continuously elevated hematocrit. Administration of crystalloid solutions (Ringer's lactate or 0.9% w/v "normal" saline) to patients with shock is usually effective in restoring circulating blood volume, but large volumes are often required. More refractory cases may require the use of colloid (Dextran 70 or the protein digest gelafundin 35,000)(58). The administration of colloids containing molecules that escape slowly from the circulation may overcome shock more quickly and may be beneficial in preventing recurrence of shock and reducing the requirement for large volume of intravenous fluid and thus the risk of fluid overload. Fluid replacement must be stopped when the hematocrit and vital signs return to normal and diuresis ensues; otherwise, pulmonary oedema will occur when the extrvasated plasma is reabsorbed(59).
Blood transfusions are contraindicated in patients with severe plasma leakage in the absence of hemorrhage, and if given, may cause pulmonary edema. Blood transfusions are indicated for patients with significant clinical bleeding(60). It may be difficult to recognize internal bleeding in the presence of hemoconcentration. A drop in hematocrit of 10% with no clinical improvement despite adequate fluid administration may indicate significant internal hemorrhage. Fresh whole blood is preferable and fresh frozen plasma and/or concentrated platelets may be indicated in some cases(61). Frequent recording of vital signs, platelet count and hematocrit determinations and monitoring urine output are important in evaluating the results of treatment(62). Prognosis depends on early recognition of shock based on careful monitoring, and proper management.
Survival of infection with one virus serotype confers lifelong immunity to re-infection with that serotype, but not to the other three serotypes. Persons can have as many as four dengue infections in their lifetime, one with each serotype.
Is isolation required for the patient? Isolation of the DHF patient is not required because there is no person-to-person transmission. It is recommended, however, that suspected DHF patients be housed in mosquito free facilities, i.e., closed buildings with air conditioning which are located in *Aedes aegypti* free grounds.

Clinical, Molecular, and Epidemiological Analysis of Dengue:

Dengue is a disease caused by four antigenically distinct but genetically related virus serotypes that cause dengue: Dengue virus (DENY 1-4)(63). It is transmitted by the *Aedes aegypti* mosquito with the clinical presentation of the disease ranging from mild forms, such as dengue fever (DF), to serious and even fatal forms. In the mild form of the disease, clinical manifestations include fever, headache, prostration, arthralgia, retro-orbital pain, nausea, rash, itchy skin, and others. The main severe form of dengue is dengue hemorrhagic fever (DHF), characterized by bleeding tendency, thrombocytopenia, and plasmatic effusion, which can progress to circulatory failure, characterizing dengue shock syndrome (DSS), and death.

The incidence of dengue has grown worldwide in recent decades. The number of people at risk is about 2.5 billion(64). A recent study estimates there to be 390 million dengue infections every year, of which 96 million manifest any level of clinical or subclinical severity. In 2007, there were over 890,000 dengue cases in America, of which 26,000 were DHF. The disease is endemic in over 100 countries in Africa, Americas, Eastern Mediterranean, Southeast Asia, and Eastern Pacific. Southeast Asia and Eastern Pacific regions are the most seriously affected. Before 1970, only nine countries had experienced DHF epidemics, a number that has increased more than four times in 1995(65). Brazil has experienced several epidemics of dengue.

Molecular Analysis:

For molecular analyses, 190 samples from patients with suspected dengue were collected from 2010 to 2013. The collection was performed from January to March of each year at the Hospital Sao Judas Tadeu or at the Centro Municipal de Apoio d Sa6de (CEMAS), both in Divinopolis, MG. The samples were collected from patients with less than 7 days of symptoms after the consent on the research project. The participants answered a questionnaire about symptoms present. This study was approved by the Research's Ethics Committee of the Universidade Federal de Sao Joao del Rei, under the identification 012/2010.

Most Frequent Symptoms

The most frequent symptoms present in the notification form from the information system for notifiable diseases are described below.

Prostration, arthralgia, exanthema, vomiting, diarrhea, bleeding, retroocular and abdominal

pain, headache, and fever may be observed.

Serological and Molecular Diagnosis:

Only 23% (946) of cases were confirmed by serological tests and the other 70,1% (2,880) were confirmed by epidemiological criteria. This information was not available for 6,9% (284) individuals. Of the 190 blood samples collected from patients with suspected dengue from 2010 to 2013, 23% were positive for DENV by molecular tests. In 2010, of the 82 blood samples collected, 22 were positive in the molecular test. The viral typing performed by nested PCR corresponded to DENV-2. This profile was found in 21 positive samples. Just one sample was positive for DENV-3. Among 22 positive cases detected by molecular diagnosis, fever was not reported by nine of these patients.

Dengue virus amplification by RT-PCR from blood samples patients. (a) Electrophoretic profile of DNA fragments (511 pb) corresponding to positive samples. A5, A6, A8: negative samples for Dengue virus. (b) Electrophoretic profile from the nested PCR for viral typing. A3, A4, A7, A8: DENV-2 fragment of 119 pb. PCR products were fractioned by 8% PAGE and visualised by silver staining. M: molecular size markers (bp).

In subsequent years, the typing of DENV has continued with DENV-3 detected in the single positive sample in 2011 from 32 samples collected and DENV-1 in one positive sample from 6 suspected cases collected in 2012. The years 2011 and 2012 had just 35 and 27 confirmed dengue cases by the serological test. In 2013, 5,998 cases were confirmed until October of this year with DENY-1 detected in 20 positive samples from 70 suspected cases collected.

Molecular diagnosis allowed the detection of DENV in samples with only one day of symptoms. Positive cases in the years analyzed were distributed as follows: five cases with one day of symptoms, 13 cases with two days of symptoms, 16 cases with three days of symptoms, five cases with four days of symptoms, one case with five days of symptoms, and one case with 6 days of symptoms. This information was missing for three patients. One patient had a negative serology but a positive molecular diagnosis and three other patients had positive serology but negative molecular diagnoses.

Economic perspective

Dengue is a viral infection transmitted by Aedes mosquitoes, with global distribution, mainly in the tropical regions. Infection with one of the four antigenically distinct dengue serotypes is often asymptomatic or mildly symptomatic, but has the potential to escalate to dengue

fever and subsequently, to life-threatening dengue hemorrhagic fever or dengue shock syndrome, and death. Although life-long immunity to the infecting serotype may develop, the more severe or life-threatening cases of dengue are more often associated with subsequent secondary infection by heterogonous dengue serotypes(66).

Contemporary global estimates from the World Health Organization suggest that 50-100 million dengue infections occur annually(67). A more recent estimate, based on cartographic modeling approaches and data from various published sources between 1960 and 2012, suggests that there are about 390 million dengue infections per year with 96 million apparent/symptomatic cases of the disease. Although the majority of dengue infections occur in Asia, there has been a dramatic increase in the number of reported dengue cases in the Americas over the last decade. Over 50 million dengue infections were estimated (using cartographic modeling approaches) in the Americas in 2010, and of these, about 40%(21.8 million infections) occurred in Brazil(68). Recent surveillance data from the Brazilian national notifiable diseases information system (SINAN; Sistema de Informacao de Agravos de Notificacao) showed that there were more than 2 million dengue cases reported in 2013, the highest annual incidence registered in Brazil since dengue surveillance was implemented in the 1980's. The increase in the incidence of dengue was probably due to the introduction of dengue serotype-4 along with the rapid spread and co-circulation of the other serotypes(69). In addition, the reporting rates may have increased due to higher dengue awareness among the population and the health workers.

Dengue can impose a significant economic and humanistic burden in countries where the disease is endemic and, as such, estimating the associated economic and disease burden can help inform policy-makers and assist them in setting priorities for disease-management strategies and for the introduction of new technologies. (Brazil, El Salvador, Guatemala, Panama, and Venezuela), and was subsequently updated as part of a later study. These previous evaluations, however, estimated the cost of dengue cases without taking into account seasonal fluctuation or costs associated with dengue outbreaks. Nonetheless, the estimated cost of dengue illness across the Americas between 2000 and 2007 was at US$2.1 billion per year with the majority of costs associated with ambulatory cases rather than hospitalized cases(70). Brazil accounted for about 40% (US$878.2 million) of the total costs in the Americas. Since these publications, recommendations and guidelines have been developed for estimating the burden and socioeconomic costs of dengue.

Statistical analysis:

A statistical analysis was performed to evaluate the differences in the clinical and laboratory manifestations among individuals with dengue from the two age groups: adults and children. For the univariate analysis, a chi-square analysis or Fisher's exact test were performed for the categorical variables. Variables with a p-value lower than 0.10 were used for a multivariate logistic regression model. p-Values lower than 0.05 were considered to be significant. A data analysis was performed with the software program SPSS 13.0 for Windows (Statistical Package for the Social Sciences, Chicago, IL, USA).

Regarding the signs and symptoms that were identified in the first evaluation at the CRD, classical signs, such as fever, headache, and myalgia, were present in over 70% of adults and children with dengue. Common clinical manifestations, such as retro-orbital pain, prostration, nausea, and vomiting, were, observed in 40-50% of adults and children.

A comparison among age groups revealed that the frequency of clinical signs and symptoms was significantly different between adults and children. The univariate analysis indicated that a headache, myalgia, anorexia, retro-orbital pain, abdominal pain, nausea, arthralgia, prostration, and bleeding were present more frequently in adults than in children. However, the multivariate analysis indicated that only myalgia (OR = 2.58; CI 95% = 2.08-3.18), retro-orbital pain (OR = 1.36; CI 95% 1.15-1.62), nausea (OR = 1.92; CI 95% = 1.60-2.30), and arthralgia (OR = 3.64; CI 95% 2.72-4.89) were significantly associated with adults with dengue. Vomiting (OR = 0.52; CI 95% = 0.43-0.61) and rash (OR = 0.46; CI 95% = 0.25-0.85) were the correlated symptoms in children in both the univariate and multivariate analyses.

Regarding the laboratory evaluations, all of the alterations (hemoconcentration, thrombocytopenia, leukopenia, and increases in ESR, AST and ALT) were more frequent in adults than in children. However, the multivariate analysis demonstrated significant differences between adults and children only for the hemo-concentration (OR = 3.04; CI 95% = 2.53-3.65), thrombocytopenia (OR = 2.17; CI 95% = 1.80-2.60), increased ESR (OR 1.81; CI 95% = 1.53-2.14), and increased ALT (OR = 3.13; CI 95% = 2.44-4.02)
Adults had a higher risk for severe dengue (7.6% vs. 3.5%) and a higher frequency of hospitalization (14.4% vs. 6.3%) than did children in both the univariate and multivariate analyses for severe dengue (OR = 1.74; Cl 95% – 1.12-2.72) and hospitalization (OR = 2.21; CI 95% = 1.59-3.06)
In this study, a higher prevalence of women were infected with dengue than of men was

detected. These data are similar to those reported by a study in Nicaragua3 and different from other studies. The children with age between 10 and 14 years old and adults between 15 and 19 years old were the age groups most prevalent affected, similar to the data reported previously. In contrast to some studies, a higher prevalence of a headache, myalgia, arthralgia, retro-orbital pain in adults were found. These findings may be related to the difficulty in identifying these signs and symptoms by the parents and the children. Abdominal pain is also more common in adults, maybe because of the anatomic-physiological differences of the organs affected by dengue according to age. There were more hemorrhagic manifestations in adults. It is believed that the primary infection acts as a protective factor against the hemorrhagic forms in children since repeated infections by different serotypes of the DENY, results in more severe manifestations.

A higher prevalence of petechiae and vomiting in children, as opposed to other studies were found.

Leukopenia and thrombocytopenia were the most prevalent laboratory findings. The induced viral destruction or the inhibition of myeloid progenitor cells causes leukopenia and peripheral destruction of platelets or destruction of megakaryocytes of the bone marrow by the virus, resulting in decreased production of platelets(71). There was no clinical correlation between thrombocytopenia and severity of Dengue. In this study, increased ALT and AST levels were observed as frequent; however, there was no correlation between these results and the severe forms of dengue. The laboratories alterations such as thrombocytopenia and elevation of ALT were more severe in adults. It is noteworthy that no respiratory and renal involvement was found.

Epidemiology of dengue:

Dengue is an acute mosquito-borne viral infection that places a significant socioeconomic and disease burden on many tropical and subtropical regions of the world. It is currently regarded as the most important arboviral disease internationally as over 50% of the world's population live in areas where they are at risk of the disease, and approximately 50% live in dengue endemic countries(72).

There are four distinct dengue virus serotypes, all of which originate from the family Flaviviridae and genus Flavivirus. The serotypes are termed DENY-1, DENY-2, DENY-3, and DENY-4, and infection with any of the four viruses results in lifelong immunity to that specific serotype. Each of the four serotypes has been individually found to be responsible for dengue epidemics and associated with more severe dengue.

Dengue disease and clinical management

Dengue is a complex disease with a wide spectrum of clinical presentations, which often goes unrecognized or is misdiagnosed as other fever-causing tropical diseases. Following the period of incubation, most patients experience a sudden onset of fever which can remain for 2-7 days and is often accompanied with symptoms such as myalgia, arthralgia, anorexia, sore throat, headaches, and a macular skin rash. It is during this period that differentiating dengue from other febrile diseases proves troublesome. The majority of people experience a self-limiting clinical course, which does not progress to the severe forms of dengue, dengue hemorrhagic fever (DHF), or dengue shock syndrome (DSS). Secondary dengue infections or particularly virulent viral strains are two factors thought to be associated with increased risk of severity. In severe cases, thrombocytopenia and increased vascular permeability can result in hemorrhagic and shock complications(73).

Dengue vector and vector control

The main arthropod vector for transmission of the dengue viruses is *Aedes aegypti*. The second, less effective vector, *Aedes albopictus* (A. albopictus), feeds on multiple species of vertebrates, but has still been shown to be responsible for some dengue transmission. Significantly, the Aedes mosquitos are predominantly active during daylight hours, which possess difficulties in controlling the vector. *Aedes aegypti* mosquitoes are now extensively spread in both the tropics and subtropics. The mosquito is renowned for its efficient 'vectorial capacity' with a high affinity for human blood, high susceptibility to the four dengue virus serotypes, and being highly adapted to urban living. A. aegypti mosquitoes breed in and around houses in regular water containers or disposed water-holding vessels. Due to this location of development and their limited flight range, female A. aegypti tend to persist in a domesticated environment. It is for this reason that humans are presumed the main cause of spread of dengue between communities. The wider prevention and control of dengue is currently reliant on vector control methods. These include environmental, biological, and chemical vector control management strategies and methodologies(74).

Dengue has been present for centuries. The first recorded symptoms compatible with dengue were noted in a Chinese medical encyclopedia in 992 AD(75), however originally published by the Chin Dynasty centuries earlier, prior to being formally edited. The disease was referred to as 'water poison' and was associated with flying insects. Epidemics that resembled dengue, with similar disease course and spread, occurred as

early as 1635 and 1699 in the West Indies and Central America, respectively. A major epidemic occurred in Philadelphia in 1780 and epidemics then became common in the USA into the early 20th century, the last outbreak occurring in 1945 in New Orleans. The viral etiology and the transmission by mosquitoes were only finally determined in the 20th century.

The origin of the primary mosquito vector, *Aedes aegypti*, is debated to be from either Africa or Asia. Regardless, by 1800 it was widespread throughout urban tropical coastal cities of the world due to the use of shipping vessels with commercial expansion(76). These shipping vessels allowed transportation of breeding sites for the vector along with humans to complete the transmission cycle, allowing for slow but evident introduction of the virus and the mosquito to coastal destinations around the world. Epidemics were spaced by 10-40 year intervals due to this shipping mode of transport(77). Expansion of the disease heightened during World War II (WWII), when troops began to disperse inland and utilize modern transportation within and between countries; thus epidemic dengue became more far-reaching.

The current global situation of dengue

Up to 3.6 billion people are estimated to now live in tropical and subtropical areas where the dengue viruses have the potential for transmission(78). Global estimates vary, but regularly approximate 50 million to 200 million dengue infections, 500,000 episodes of severe dengue (DHF/DSS), and over 20,000 dengue related deaths occur annually(79). In 2012, dengue was once again classified by the World Health Organization (WHO) as the 'most important mosquito-borne viral disease in the world' due to significant geographic spread of the virus and its vector into previously unaffected areas and the subsequent costly burden of disease it brings(80). Diaz-Quijano and Waldman conducted an ecological study investigating the determinants of the dengue mortality burden. Length of recognized endemicity, rainfall, and population density were all shown to be associated with dengue mortality in Latin America and the Caribbean(81).

A study which reviewed all nations in the Americas (with available data from the PAHO, 2000-2007) estimated an aggregate annual cost of dengue for the Americas at US$2.1billion(82). Approximately 60% of this cost related to indirect or 'productivity' losses, and the figure notably excluded prevention costs. A study of twelve countries in South East Asia using available data from 2001-2010 showed an aggregate annual economic burden of US$950 million amongst the studied nations, with approximately 52% of these costs coming from productivity loss(83). Due to poor disease surveillance, low level of reporting, low case

fatality rate, difficulties in diagnosis, and inconsistent comparative analyses, the true incidence and impact of dengue is likely significantly higher than that which is currently reported. Thus, the true global burden of disease and associated economic impact is unknown. However, Brady et al have conducted the first of a series of steps in evidence consensus mapping of global dengue incidence to better determine the population at risk(84). Their 2012 publication suggested an `upper bound' total of 3.97 billion people living in 128 countries are at risk of dengue globally, 824 million in urban residences, and 763 in peri-urban residences. The same group published again in April 2013 using cartographic approaches. These data suggested 390 million dengue infections occur annually worldwide, including both apparent and inapparent infections, almost double the highest figure regularly reported to date.

Despite the level of uncertainty on total numbers, every WHO region now has dengue transmission and that there are more than 125 dengue endemic countries worldwide(85).

WHO Southeast Asia region

Almost 75% of the global population exposed to dengue lives in Asia-Pacific. 1.3 billion of these at-risk individuals live in ten dengue endemic countries in SEA, and dengue is a leading cause of hospitalization and death in children from the region(86). The rates of the disease reported in each of the SEA countries varies as they include either laboratory confirmed, probable, or suspected cases. However, it is clearly evident from data collated by WHO that, in SEA, an overall expansion of dengue has occurred over the last decade. In 2003, eight countries in SEA had reported cases of dengue and, by 2009, all SEA member countries excluding the Democratic People's Republic of Korea reported indigenous cases. Epidemics continue to persist on regular 3-5 year cycles throughout SEA, and the number of reported cases continues to increase along with the severity of cases in many member countries.187,333 dengue cases were reported to WHO in 2010 from the region. Eight SEA countries are now also classified as hyperendemic with all four of the dengue virus serotypes present. Severe dengue is endemic in most SEA countries, with rates of severe dengue being 18 times higher in this region compared with the Americas(87).

WHO Western Pacific region

The WHO Western Pacific and SEA regions combined are attributed 75% of the global dengue disease burden. The number of reported cases of dengue has increased continuously over the past decade in the Western Pacific. 353,907 dengue cases and 1073 deaths were reported in the region as a whole in 2010(88).

In the Asian subregion of the WHO Western Pacific region, the greatest burden of dengue

currently originates from Cambodia, the Lao People's Democratic Republic, Malaysia, the Philippines, Singapore, and Vietnam(81). The number of reported cases increased in each of these nations over the past ten years, and all four serotypes have been identified in these high disease-burden settings. From the Pacific subregion, 91% of reported cases came from French Polynesia, New Caledonia, Vanuatu, and Australia. Two dengue virus serotypes were reintroduced from the Americas to the Pacific Islands in 1964 (DENY-3) and in 1971 following a 25 year absence. The next decade saw gradual introduction of all four serotypes from Asia, which remain in circulation today. As a result, island nations in the Pacific show a particular susceptibility to dengue epidemics and severe dengue. In Australia, dengue activity, including indigenous outbreaks, occur in Northern Queensland where *Aedes aegypti* is present. In 2009 and 2010, more than 1000 cases of dengue were reported in Australia(82).

WHO region of the Americas

Despite the absence of dengue transmission in the middle of the 20th century, almost all countries in the Americas now have hyperendemicity with indigenous dengue transmission. Epidemics occur cyclically in the region every three to five years, as they do in SEA, with increasing frequency and size, particularly in Latin America. In 2010, more than 1.6 million cases of dengue were reported in the Americas alone, 49,000 of these being severe dengue. Only two countries in Latin America remain to be without indigenous transmission, Uruguay and Continental Chile. Locally acquired cases of dengue have also now been reported in the USA.. The 'Integrated Management Strategy for Dengue Prevention' is striving to reduce the disease and economic burden that dengue places currently in the Americas.

WHO African region

Little has been known or reported about the situation in Africa amidst the geographic spread of dengue worldwide. Despite dengue not being officially reported to WHO by African countries and the probable under-recognition of dengue, evidence suggests that outbreaks are increasing in size and frequency in the region. Available outbreak data suggest 22 African countries reported sporadic cases or outbreaks between 1960 and 2010(83). Amarasinghe et al 13 conducted a review of existing databases and literature in 2011 that showed dengue transmission is endemic in 34 countries in the African region. 22 of these countries had local disease transmission, 20 reports of lab-confirmed cases, and two reports of clinical cases alone. No 'local' reports of dengue occurred in the remaining countries, All dengue virus serotypes have been seen in Africa, and DENY-2 appears to have caused most epidemics(84). Due to the significant endemicity of malaria throughout the African region, the majority (>70%) of 'febrile illnesses', including dengue, are likely to be misdiagnosed and treated as

malaria. This negatively impacts attempts to draw a comprehensive picture of the epidemiology of dengue in the region and establish regular surveillance, outbreak monitoring, and relevant prevention and control activities.

WHO European region

The last reported epidemic of dengue in Europe was between 1926 and 1928 in Greece. This epidemic implicated *Aedes aegypti* as the predominant vector and saw high mortality of cases(85). No dengue transmission had been reported since this time until *Aedes albopictus* became established in Europe in the 1990s as a result of increasing global trade of used tires. Today, there is a very real and apparent threat of dengue outbreaks in Europe. Imported cases in travelers are seen frequently and, in 2010, local transmission of dengue was reported in both Croatia and France. The Madeira Islands of Portugal have been in the midst of an outbreak since October 2012. This outbreak had resulted in 2164 cases by February 2013, with 78 imported cases from recent travelers to Madeira detected in 13 other countries throughout Europe. Thus, despite Europe being free of dengue for the majority of the 20th century, the global expansion of dengue is finally impacting the region.

WHO Eastern Mediterranean region

In the Eastern Mediterranean region, dengue is classified as an 'emerging discase'. Cases have only been officially reported to WHO for the last 2 decades, during which time three countries – Saudi Arabia, Pakistan, and Yemen – have had multiple outbreaks.6 For example, in 2011, the city of Lahore in Pakistan experienced a major dengue outbreak associated with 21,685 confirmed cases and 350 deaths, mainly due to DENY-2.Smaller outbreaks involving multiple serotypes of dengue virus are now being reported more frequently from countries such as Sudan, Djibouti, and Somalia. This highlights the probable geographic expansion of dengue within the Eastern Mediterranean Region, as with elsewhere globally.

Dengue in the future

Many experts hypothesize that dengue will increase in the future, including geographic expansion, incidence and reporting to WHO. It is therefore important to elaborate on some of the potential factors that drive dengue activity, as well as the global strategic direction to address this growth.

Viral evolution

Dengue viruses have been cataloged as having a low, medium, or high epidemiological impact according to their likelihood for human transmission and the clinical severity of dengue epidemics. In other words, some viruses largely prevail in syllabic cycles among non-

human primate populations, rarely transmitting to humans, while others are the viral agents causing mild dengue fever(86). There are some DENY-2 and DENY-3 genotypes found more commonly in the Americas which are known to be comparatively less virulent than Asian genotypes of the same serotype, as evidenced by the reduced growth in both mosquitoes and culture. Wang et a155 demonstrated that domain III may play a role in viral adaptation to naive hosts, whether mosquito or human, through analysis of modifications to the envelope protein postulated to correlate with endemic and/or epidemic emergence. Genotypes with greater virulence are driving out virus strains of lesser epidemiological impact.

Climate change factors

Temperature is known to play a role in adult vector survival, viral replication, and infective periods. Increases of temperature may result in increased survival and or migration of vectors into previously non-endemic geographic areas outside the tropics. As the proliferation of Aedes mosquitoes is climate dependent, climate or meteorological factors can potentially provide useful information in predictive models. Weather variability has shown to be predictive of dengue activity. According to the Intergovernmental Panel on Climate Change, mean temperatures are predicted to rise globally(87). This may create climatic and environmental conditions conducive to the proliferation of Aedes species in areas that are currently non-endemic. The climatic suitability of many currently non-endemic areas and climatic similarity with endemic areas suggests that both *Aedes aegypti* and *Aedes albopictus* could become established or reestablished in the near future. A study conducted in the Southwest Pacific suggested that global temperature increases observed over the last four decades corresponded with an increased risk of dengue outbreaks(88). Some studies on climate change and dengue show a possible increase in transmission due to higher temperatures, humidity, and precipitation associated with changes in climate. This supports the notion that observed climatic changes, including increased average global temperature and increased humidity, theoretically increase the epidemic potential of dengue.

The individual role climate change plays in the last decades' resurgence of dengue remains uncertain and is an area of current modeling research. Some authors also argue against climate as the main driver for dengue expansion. Beebe et al concluded from their Southeast Australian study that an increased risk of *Aedes aegypti* range expansion in Australia was due to the human adaptation of installing large domestic water storing containers as a response to persisting regional drying, rather than due to climate change itself. Furthermore,

dengue and yellow fever caused multiple epidemics in the southern parts of the USA in the 18th, 19th, and early 20th centuries. Their eventual control was not due to a change in climate, but rather due to changes with industrialization and modernization.

Globalization, travel, and trade factors

While climate change alone may not be a comprehensive and sufficient causal factor in the current and ongoing expansion of dengue, broader 'global change' may be. The `global change' framework seeks to account for multiple factors of the modern world contributing to vector-borne communicable disease. Modern contributing factors to the rapid expansion of vector-borne communicable disease include globalization factors, such as travel and trade, associated with vector accommodating trends in modern human settlement and suitable climate conditions. The contributions of increased mobility, both of vector and human populations, may be the most important variable to explain the recent increase in dengue transmission.

Climate and human settlement factors may enable and explain the risk of introduction or reintroduction of dengue into non-endemic zones where they border areas of endemic transmission. For other areas, further outside the tropics, the slight expansion due to climate change and human-vector interactions pale in comparison to factors of globalization. Globalization has been a main contributor and result of recent global economic development, creating a global ecosystem of exchange. The current global reality is one of international passenger travel and intercontinental exchange of goods. By 2011, passenger air travel saw a 40-fold increase compared to the middle of the 20th century with ever increasing travel to and from dengue endemic areas. Human travel by those infected with dengue is thought to be the main driver of global transmission and expansion of the disease(89). Modern transport accounts for the importation of dengue by overcoming natural barriers of travel time and geography, which had previously limited expansion from endemic areas into non-endemic areas. A recent model on the geospatial distribution of transmission via passenger air travel identified routes on which importation of dengue was an increased risk. Increased risk routes between the USA and Latin America, and also between Europe and Asia, were identified, ranked, and correlated with the increased geographical distribution of the secondary dengue vector, *Aedes albopictus*. Intercontinental air travel between areas within the tropics has resulted in transmission of all four dengue virus serotypes in some areas. Overcrowded airports located in the tropics function as the ideal urban breeding ground and distribution point for dengue viruses within and outside current areas of transmission.

Further globalization factors, which are contributing to the expansion of dengue transmission

and risk of importation of dengue, include not only travel, but also trade. International transport of cargo and goods, especially via commercial sea shipment, can also export and import dengue's primary and secondary vectors, *Aedes aegypti* and *Aedes albopictus*, respectively. Transatlantic transport of used auto tires has been linked with the introduction of exotic American mosquito varieties into Italy, which contributed to other vector-borne disease epidemics(90). Given the vectors' suitability to breed and survive sea travel within water collected in a tire, their transport has contributed to a major public health threat in the last few decades, and this will only increase as more automobiles are consumed globally.

Settlement factors

Human factors, including both urban and rural settlement patterns, contribute to currently observed trends of increased incidence and expansion of dengue transmission(91). Rapid urbanization and population growth have been identified as strong contributing factors to the increase of global dengue transmission and geographic expansion. These two factors, particularly in low- and middle-income countries in tropical and subtropical regions, often precede the construction of necessary infrastructures for safe and comprehensive collection, storage, and disposal of water. Urban and suburban development may also provide new man-made breeding sites in the built environment, prior to human inhabitants occupying them. In this manner, rapid urbanization facilitates the creation of urban breeding sites for the most potent dengue vector, *Aedes aegypti*. It thrives in urban environments in that the mosquito breeds preferentially in the artificial containers often used in urban water collection. The increased density of both mosquito and human populations, as part of urban population growth, compounds this effect in terms both of vector suitability and transmission of dengue. While current research and policy interventions often treat dengue expansion as a phenomenon associated with urban human settlement, the incidence of the disease in rural areas is also on the rise. Some studies suggest that rural dengue incidence can even surpass urban and semi-urban communities within the same region(92).

Previous and ongoing underestimation of rural incidence may be attributable to similar vector-suitable breeding sites between some regions' urban-poor and rural communities. In addition, growing interconnectivity between rural and urban areas via increasing road infrastructure, combined with decreased access to diagnostics and surveillance may act as a silent conduit for rural dengue transmission and greater underestimation of rural incidence compared to more urban areas. The significant role and mechanisms of human involvement in creating a conducive ecology for dengue transmission, in addition to climate

environmental factors, is being increasingly considered and modeled in current research.

Socioeconomic factors

Historical dengue incidence and decline in Europe and the US, among other areas, suggests the role of socioeconomic development on dengue transmission and control(93). Multiple studies compared dengue endemicity and seroprevalence between neighboring border cities in Northern Mexico and Southern Texas. These highlights the importance of socioeconomic factors on the transmission of dengue, where climatic suitability was similar. In one such comparative cross-sectional study from 2004, current dengue seroprevalence was found to be 7.3% in Matamoros, Mexico, but only 2% in Brownsville, Texas, just across the border in the USA(94). Another similar serosurvey in 2005 suggested an even greater disparity between dengue incidence in Matamoros and Brownsville, reporting current dengue infection in 32% and 4%, respectively, of the 273 reporting study participants and estimating past dengue infection prevalence in 77% and 39%, respectively(95). Key similarities observed among both cities included climate and geography, vector mosquito habitat and density, and human host social factors, for instance household size, use of insect screens, and basic sanitation. Socioeconomic and behavioral factors including income, water storage, usage of air-conditioning, waste disposal, and cross-border travel differed sustainably, as did dengue prevalence. In endemic areas, including the USA–Mexico border, more favorable socioeconomic factors resulting in higher utilization of air conditioning and domestic screening, as well as improved water and waste disposal infrastructure, are recommended to reduce larvae breeding sites and dengue transmission. Environmental management that aims to reduce, remove, and displace breeding sites from urban areas is recognized as a key mechanism to control dengue transmission.

Global strategic direction

In light of the potential for continued expansion of dengue globally, it is essential to reflect on policy and strategic direction that attempts to reduce the impact of this disease. Dengue has been classified as a 'neglected topical disease,' based on the historical lack of coordinated efforts, political will, and research attention despite the significant disease, social, and economic burden it places internationally. The different classification has encouraged prioritization of dengue via the WHO's Global Strategy for Dengue Prevention and Control, 2012-2020. The overall goal of this multi-sect oral strategy is `to reduce the burden of dengue(96). The document also defines objectives, technical elements, and enabling factors for effective implementation such as advocacy, partnership, coordination, and collaboration.

The need to gain improved dengue disease burden estimates is one of three key objectives identified for dengue control with a timeframe for completion of 2015. More accurate epidemiological and surveillance data will enable further political prioritization for the currently 'neglected' disease(97). It would also enable improved decision making and rational allocation of financial, research, and other resources to the areas of greatest need. For example, epidemiological data will be essential in planning funding, allocation, and distribution of dengue vaccines that could potentially become available in the next decade.

Sustainable vector control is one technical element of the Global Strategy for Dengue Prevention and Control, 2012-2020(98). In light of limited therapeutic strategies and the current lack of a vaccine, effective vector control methods are an essential component of the strategic direction to reduce dengue mortality and morbidity by 2020. Integrated Vector Management (IVM) is the strategic approach promoted to Countries by the WHO as a rational, cost-effective, and optimal decision-making process for vector control programs. For dengue vectors, this involves using a combination of approaches incorporating key elements of social mobilization, integration of chemical and non-chemical control methods targeting areas of high human-vector contact, evidence based decision-making guiding research and policy, as well as capacity building. Utilizing an effective integrated vector control strategy will aid in reduction of dengue transmission.

Some researchers suggest dengue prioritization has now evolved and query how long it will be classified as 'neglected' disease. Furthermore, the impact of dengue has now progressed beyond those living in poverty as wealthier urbanized populations also have endemic dengue. Whilst this expands the experience and relevance of dengue to a broader group, the resulting impact on dengue control and future epidemiology is currently unknown. Irrespective of the poverty-promoting aspects of dengue, such as reduced economic potential with days off school and work, persist in the majority of dengue endemic settings. Perhaps, with continued expansion alongside improved epidemiological information, further prioritization and coordination of resources will be encouraged and we may see the objectives of the WHO 'Global Strategy' met by 2020.

Conclusion

Dengue is now endemic in more than 125 countries globally. Reasons for the currently observed and predicted expansion are multi-factorial. They may include climate change, virus evolution, and societal factors such as rapid urbanization, population growth and development, socioeconomic factors, as well as global travel and trade. There is no antiviral therapy or vaccination available for dengue at this time, leaving only early detection and

symptomatic treatment with fluid resuscitation essential for management of severe cases. As a result of limited therapeutic strategies, effective vector control methods are essential and are therefore promoted globally by the WHO through the strategic approach of IVM for dengue, this approach targets the *Aedes* genus of mosquito in settings where high levels of human-vector contact occur The WHO Global Strategy for Dengue Prevention and Control, 2012-2020, highlights the need for improved estimates of the true burden of dengue disease globally due to the currently presumed under-representation. Surveillance and reporting is paramount for effective dengue control, and more accurate quantification of the impact of dengue globally will allow improved political, financial, and research prioritization as well as informed decision making and enhanced modeling. The DENVs are old viruses that have re-emerged during the latter half of the 20th century. Regarded as a tropical fever disease affecting more than two thirds of the world's population, dengue is also the main cause after malaria of tropical fever among travelers and ranks as the most important mosquito-borne viral disease in the world. The lack of potent antiviral drugs and an effective vaccine results in 500,000 individuals, mainly children, being hospitalized with severe dengue every year and causes tremendous economic losses for both households and whole nations. The pathogenesis of the DENVs are not well understood, partly due to the absence of good animal models. Effective vector control measures are the sole weapon against dengue today, while we are hoping for improved diagnostics, clinical treatment, and an effective vaccine. The known social, economic, and disease burden of dengue internationally is alarming and it is evident that the wider impact of this disease is grossly underestimated. An international multi-sectored response, such as that outlined in the WHO Global Strategy for Dengue Prevention and Control, 2012-2020, is now essential to reduce the significant influence of disease projects globally.

i

1. Tuiskunen Bäck, A., & Lundkvist, Å. (2013). Dengue viruses–an overview. *Infection ecology & epidemiology*, *3*(1), 19839.

2. Tuiskunen Bäck, A., & Lundkvist, Å. (2013). Dengue viruses–an overview. *Infection ecology & epidemiology*, *3*(1), 19839.

3. Welsch, S., Miller, S., Romero-Brey, I., Merz, A., Bleck, C. K., Walther, P., ... & Bartenschlager, R. (2009). Composition and three-dimensional architecture of the dengue virus replication and assembly sites. *Cell host & microbe*, *5*(4), 365-375.

4. Grard, G., Moureau, G., Charrel, R. N., Lemasson, J. J., Gonzalez, J. P., Gallian, P., ... & de Lamballerie, X. (2007). Genetic characterization of tick-borne flaviviruses: new insights into evolution, pathogenetic determinants and taxonomy. *Virology*, *361*(1), 80-92.

5. Dey, L., & Mukhopadhyay, A. (2017). DenvInt: A database of protein–protein interactions between dengue virus and its hosts. *PLoS neglected tropical diseases*, *11*(10), e0005879.

6. Halstead, S. B. (2000). Global Perspectives on Dengue Research.

7. Halstead, S. B. (1970). Observations related to pathogensis of dengue hemorrhagic fever. VI. Hypotheses and discussion. *The Yale journal of biology and medicine*, *42*(5), 350.

8. Messina, J. P., Brady, O. J., Scott, T. W., Zou, C., Pigott, D. M., Duda, K. A., ... & Simmons, C. P. (2014). Global spread of dengue virus types: mapping the 70 year history. *Trends in microbiology*, *22*(3), 138-146.

9. World Health Organization. (2011). Comprehensive guideline for prevention and control of dengue and dengue haemorrhagic fever.

10. Halstead, S. B. (2000). Global Perspectives on Dengue Research.

11. Halstead, S. B. (2000). Global Perspectives on Dengue Research.

12. World Health Organization. (2011). Comprehensive guideline for prevention and control of dengue and dengue haemorrhagic fever.

13. Gubler, D. J. (1998). Dengue and dengue hemorrhagic fever. *Clinical microbiology reviews*, *11*(3), 480-496.

14. Hammon, W. M. (1973). Dengue Hemorrhagic Fever—Do we know its cause?. *The American journal of tropical medicine and hygiene*, *22*(1), 82-91.

15. Kouri, G. P., Guzmán, M. G., Bravo, J. R., & Triana, C. (1989). Dengue haemorrhagic fever/dengue shock syndrome: lessons from the Cuban epidemic, 1981. *Bulletin of the World Health Organization*, *67*(4), 375.

16. Halstead, S. B. (1980). Dengue haemorrhagic fever—a public health problem and a field for research. *Bulletin of the World Health Organization*, *58*(1), 1.

17. Halstead, S. B. (1989). Antibody, macrophages, dengue virus infection, shock, and hemorrhage: a pathogenetic cascade. *Reviews of infectious diseases*, *11*(Supplement_4), S830-S839.

18. Halstead, S. B. (2000). Global Perspectives on Dengue Research.

19. Downs, W. G. (1982). The Rockefeller Foundation virus program: 1951-1971 with update to 1981. *Annual review of medicine*, *33*(1), 1-30.

20. Halstead, S. B. (2000). Global Perspectives on Dengue Research.

21. Utzinger, J., Tozan, Y., & Singer, B. H. (2001). Efficacy and cost-effectiveness of environmental management for malaria control. *Tropical Medicine & International Health*, *6*(9), 677-687.

22. Vasconcelos, P. F., Rosa, A. P., Pinheiro, F. P., Rodrigues, S. G., Rosa, E. S., Cruze, A. C., & Rosa, J. F. (1999). Aedes aegypti, Dengue and Re-urbanization of Yellow Fever in Brazil and other South American Countries-Past and Present Situation and Future Presentative.

23. Halstead, S. B. (2000). Communicable Diseases Dengue/DHF Dengue Bulletin Volume 24, December-2000 Global Perspectives on Dengue Research. *Dengue Bulletin*, *24*.

24. Wan, S. W., Lin, C. F., Wang, S., Chen, Y. H., Yeh, T. M., Liu, H. S., ... & Lin, Y. S. (2013). Current progress in dengue vaccines. *Journal of biomedical science*, *20*(1), 37.

25. Bhamarapravati, N., & Sutee, Y. (2000). Live attenuated tetravalent dengue vaccine. *Vaccine*, *18*, 44-47.

26. Halstead, S. B. (2000). Communicable Diseases Dengue/DHF Dengue Bulletin Volume 24, December-2000 Global Perspectives on Dengue Research. *Dengue Bulletin*, *24*.

27. Jansen, C. C., & Beebe, N. W. (2010). The dengue vector Aedes aegypti: what comes next. *Microbes and infection*, *12*(4), 272-279.

28. Gubler, D. J. (2014). Dengue viruses: their evolution, history and emergence as a global public health problem. *Dengue and dengue hemorrhagic fever. 2nd ed. London*, 1-29.

29. Boyer, S., Calvez, E., Chouin-Carneiro, T., Diallo, D., & Failloux, A. B. (2018). An overview of mosquito vectors of Zika virus. *Microbes and infection*.

30. Sultana, A. (2014). " Eight to ten years ago, dengue was a killer disease... but it is not a killer disease anymore:" a qualitative approach to dengue in Dhaka, Bangladesh.

31. Afroze, A. I. N. U. N., Chowdhury, M. A. J., Kibria, G., Saha, R. K., Jalil, M. A., & Khan, M. A. (2002). Clinical profile and outcome of dengue patients during the first outbreak in Dhaka-a hospital based study. *Bangl J Med*, *13*, 16-20.

32. Farhana, R., Awatef, K. F., Khanum, H., & Akter, T. (2014). Prevalence of dengue fevers among the patients of different economic status attended at local hospital in Dhaka. *Bangladesh Journal of Zoology*, *42*(2), 161-168.

33. Gubler, D. J. (1998). Epidemic dengue and dengue hemorrhagic fever: a global public health problem in the 21st century. In *Emerging infections 1* (pp. 1-14). American Society of Microbiology.

34. World Health Organization. (1999). *Prevention and control of dengue and dengue haemorrhagic fever* (No. Regional Publication No. 29). WHO Regional Office for South-East Asia.

35. Mackenzie, J. S., Gubler, D. J., & Petersen, L. R. (2004). Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. *Nature medicine*, *10*(12s), S98.

36. World Health Organization. (1999). *Prevention and control of dengue and dengue haemorrhagic fever* (No. Regional Publication No. 29). WHO Regional Office for South-East Asia.

37. Reeves, W. C. (1965). Ecology of mosquitoes in relation to arboviruses. *Annual review of entomology*, *10*(1), 25-46.

38. Gubler, D. J., & Rosen, L. (1976). Variation among geographic strains of Aedes albopictus in suceptibility to infection with dengue viruses. *The American journal of tropical medicine and hygiene*, *25*(2), 318-325.

39. Gubler, D. J. (1998). Dengue and dengue hemorrhagic fever. *Clinical microbiology reviews*, *11*(3), 480-496.

40. Gubler, D. J. (1998). Epidemic dengue and dengue hemorrhagic fever: a global public health problem in the 21st century. In *Emerging infections 1* (pp. 1-14). American Society of Microbiology.

41. Halstead, S. B. (1989). Antibody, macrophages, dengue virus infection, shock, and hemorrhage: a pathogenetic cascade. *Reviews of infectious diseases*, *11*(Supplement_4), S830-S839.

42. Huerre, M. R., Lan, N. T., Marianneau, P., Hue, N. B., Khun, H., Hung, N. T., ... & Deubel, V. (2001). Liver histopathology and biological correlates in five cases of fatal dengue fever in Vietnamese children. *Virchows Archiv*, *438*(2), 107-115.

43. Martina, B. E., Koraka, P., & Osterhaus, A. D. (2009). Dengue virus pathogenesis: an integrated view. *Clinical microbiology reviews*, *22*(4), 564-581.

44. El-Badry, A. A., & Al Ali, K. H. (2010). Prevalence and seasonal distribution of dengue mosquito, Aedes aegypti (Diptera: Culicidae) in Al-Madinah Al-Munawwarah.

45. Rozendaal, J. A. (1997). *Vector control: methods for use by individuals and communities*. World Health Organization.

46. World Health Organization. (2003). Guidelines for dengue surveillance and mosquito control.

47. Soares, C. N., Faria, L. C., Peralta, J. M., De Freitas, M. R. G., & Puccioni-Sohler, M. (2006). Dengue infection: neurological manifestations and cerebrospinal fluid (CSF) analysis. *Journal of the neurological sciences*, *249*(1), 19-24.

48. Kurane, I. (2007). Dengue hemorrhagic fever with special emphasis on immunopathogenesis. *Comparative immunology, microbiology and infectious diseases*, *30*(5-6), 329-340.

49. Gubler, D. J. (1998). Dengue and dengue hemorrhagic fever. *Clinical microbiology reviews*, *11*(3), 480-496.

50. Nelson, E. R., & Bierman, H. R. (1964). Dengue fever: a thrombocytopenic disease?. *Jama*, *190*(2), 99-103.

51. Gourinat, A. C., O'Connor, O., Calvez, E., Goarant, C., & Dupont-Rouzeyrol, M. (2015). Detection of Zika virus in urine. *Emerging infectious diseases*, *21*(1), 84.

52. Kalayanarooj, S. (2011). Clinical manifestations and management of dengue/DHF/DSS. *Tropical medicine and health*, *39*(4SUPPLEMENT), S83-S87.

53. De Paula, S. O., & Fonseca, B. A. L. D. (2004). Dengue: a review of the laboratory tests a clinician must know to achieve a correct diagnosis. *Brazilian Journal of Infectious Diseases*, *8*(6), 390-398.

54. Guzman, M. G., Halstead, S. B., Artsob, H., Buchy, P., Farrar, J., Gubler, D. J., ... & Nathan, M. B. (2010). Dengue: a continuing global threat. *Nature Reviews Microbiology*, *8*(12supp), S7.

55. Guzmán, M. G., & Kourí, G. (2004). Dengue diagnosis, advances and challenges. *International journal of infectious diseases*, *8*(2), 69-80. Rohde, J. E. (1978). Clinical management of severe dengue. *Tropical doctor*, *8*(2), 54-61.

56. Dung, N. M., Day, N. P. J., Tam, D. T. H., Loan, H. T., Chau, H. T. T., Minh, L. N., ... & White, N. J. (1999). Fluid replacement in dengue shock syndrome: a randomized, double-blind comparison of four intravenous-fluid regimens. *Clinical Infectious Diseases*, *29*(4), 787-794.

57. World Health Organization. (1999). Guidelines for treatment of dengue fever.

58. Nimmannitya, S. (1993). Management of dengue and dengue haemorrhagic fever. *Monograph on dengue/dengue haemorrhagic fever. World Health Organization Regional Office for South-East Asia, New Delhi, India*, 55-61.

59. World Health Organization. (1999). *Prevention and control of dengue and dengue haemorrhagic fever* (No. Regional Publication No. 29). WHO Regional Office for South-East Asia.

60. Nimmannitya, S. (1993). Management of dengue and dengue haemorrhagic fever. *Monograph on dengue/dengue haemorrhagic fever. World Health Organization Regional Office for South-East Asia, New Delhi, India*, 55-61.

61. Monath, T. P. (1994). Dengue: the risk to developed and developing countries. *Proceedings of the National Academy of Sciences*, *91*(7), 2395-2400. World Health Organization. (1999). Guidelines for treatment of dengue fever.

62. Guzman, A., & Istúriz, R. E. (2010). Update on the global spread of dengue. *International journal of antimicrobial agents*, *36*, S40-S42.

63. Gubler, D. J. (1998). Dengue and dengue hemorrhagic fever. *Clinical microbiology reviews*, *11*(3), 480-496.

64. Weaver, S. C., & Vasilakis, N. (2009). Molecular evolution of dengue viruses: contributions of phylogenetics to understanding the history and epidemiology of the preeminent arboviral disease. *Infection, genetics and evolution*, *9*(4), 523-540.

65. Bhatt, S., Gething, P. W., Brady, O. J., Messina, J. P., Farlow, A. W., Moyes, C. L., ... & Myers, M. F. (2013). The global distribution and burden of dengue. *Nature*, *496*(7446), 504.

66. Martelli, C. M. T., Junior, J. B. S., Parente, M. P. P. D., Zara, A. L. D. S. A., Oliveira, C. S., Braga, C., ... & Mendes, M. C. O. (2015). Economic impact of dengue: multicenter study across four Brazilian regions. *PLoS neglected tropical diseases*, *9*(9), e0004042.

67. Nunes, M. R. T., Faria, N. R., Vasconcelos, H. B., de Almeida Medeiros, D. B., de Lima, C. P. S., Carvalho, V. L., ... & Rodrigues, S. G.

(2012). Phylogeography of dengue virus serotype 4, Brazil, 2010–2011. *Emerging infectious diseases*, *18*(11), 1858.

68. Shepard, D. S., Coudeville, L., Halasa, Y. A., Zambrano, B., & Dayan, G. H. (2011). Economic impact of dengue illness in the Americas. *The American journal of tropical medicine and hygiene*, *84*(2), 200-207.

69. Lin, S. F., Liu, H. W., Chang, C. S., Yen, J. H., & Chen, T. P. (1989). Hematological aspects of dengue fever. *Gaoxiong yi xue ke xue za zhi= The Kaohsiung journal of medical sciences*, *5*(1), 12-16.

70. Murray, N. E. A., Quam, M. B., & Wilder-Smith, A. (2013). Epidemiology of dengue: past, present and future prospects. *Clinical epidemiology*, *5*, 299.

71. Wills, B. A., Oragui, E. E., Stephens, A. C., Daramola, O. A., Dung, N. M., Loan, H. T., ... & Levin, M. (2002). Coagulation abnormalities in dengue hemorrhagic fever: serial investigations in 167 Vietnamese children with dengue shock syndrome. *Clinical infectious diseases*, *35*(3), 277-285.

72. Murray, N. E. A., Quam, M. B., & Wilder-Smith, A. (2013). Epidemiology of dengue: past, present and future prospects. *Clinical epidemiology*, *5*, 299.

73. Wu, X., Lang, L., Ma, W., Song, T., Kang, M., He, J., ... & Ling, L. (2018). Non-linear effects of mean temperature and relative humidity on dengue incidence in Guangzhou, China. *Science of The Total Environment*, *628*, 766-771.

74. Murray, N. E. A., Quam, M. B., & Wilder-Smith, A. (2013). Epidemiology of dengue: past, present and future prospects. *Clinical epidemiology*, *5*, 299.

75. Murray, N. E. A., Quam, M. B., & Wilder-Smith, A. (2013). Epidemiology of dengue: past, present and future prospects. *Clinical epidemiology*, *5*, 299.

76. Wilder-Smith, A., Renhorn, K. E., Tissera, H., Abu Bakar, S., Alphey, L., Kittayapong, P., ... & Rocklöv, J. (2012). DengueTools: innovative tools and strategies for the surveillance and control of dengue. *Global health action*, *5*(1), 17273.

77. Murray, N. E. A., Quam, M. B., & Wilder-Smith, A. (2013). Epidemiology of dengue: past, present and future prospects. *Clinical epidemiology*, *5*, 299.

78. Lourenço, J., & Recker, M. (2014). The 2012 Madeira dengue outbreak: epidemiological determinants and future epidemic potential. *PLoS neglected tropical diseases*, *8*(8), e3083.

79. Díaz-Quijano, F. A., & Waldman, E. A. The American Society of Tropical Medicine and Hygiene. *Am. J. Trop. Med. Hyg*, *86*(2), 328.

80. Shepard, D. S., Coudeville, L., Halasa, Y. A., Zambrano, B., & Dayan, G. H. (2011). Economic impact of dengue illness in the Americas. *The American journal of tropical medicine and hygiene*, *84*(2), 200-207.

81. Murray, N. E. A., Quam, M. B., & Wilder-Smith, A. (2013). Epidemiology of dengue: past, present and future prospects. *Clinical epidemiology*, *5*, 299.

82. Brady, O. J., Gething, P. W., Bhatt, S., Messina, J. P., Brownstein, J. S., Hoen, A. G., ... & Hay, S. I. (2012). Refining the global spatial limits of dengue virus transmission by evidence-based consensus. *PLoS neglected tropical diseases*, *6*(8), e1760.

83. Murray, N. E. A., Quam, M. B., & Wilder-Smith, A. (2013). Epidemiology of dengue: past, present and future prospects. *Clinical epidemiology*, *5*, 299.

84. Murray, N. E. A., Quam, M. B., & Wilder-Smith, A. (2013). Epidemiology of dengue: past, present and future prospects. *Clinical epidemiology*, *5*, 299.

85. Halstead, S. B. (2006). Dengue in the Americas and Southeast Asia: do they differ?. *Revista panamericana de salud publica*, *20*, 407-415.

86. Murray, N. E. A., Quam, M. B., & Wilder-Smith, A. (2013). Epidemiology of dengue: past, present and future prospects. *Clinical epidemiology*, *5*, 299.

87. Murray, N. E. A., Quam, M. B., & Wilder-Smith, A. (2013). Epidemiology of dengue: past, present and future prospects. *Clinical epidemiology*, *5*, 299.

88. Hu, W., Clements, A., Williams, G., Tong, S., & Mengersen, K. (2011). Spatial patterns and socioecological drivers of dengue fever transmission in Queensland McCarthy, J. J., Canziani, O. F., Leary, N. A., Dokken, D. J., & White, K. S. (Eds.). (2001). *Climate change 2001: impacts, adaptation, and vulnerability: contribution of Working Group II to the third assessment report of the Intergovernmental Panel on Climate Change* (Vol. 2). Cambridge University Press.

89. Murray, N. E. A., Quam, M. B., & Wilder-Smith, A. (2013). Epidemiology of dengue: past, present and future prospects. *Clinical epidemiology*, *5*, 299.

90. Murray, N. E. A., Quam, M. B., & Wilder-Smith, A. (2013). Epidemiology of dengue: past, present and future prospects. *Clinical epidemiology*, *5*, 299.

91. Banu S, Hu W, Hurst C, Tong S. Dengue transmission in the Asia-Pacific region: impact of climate change and socio-environmental factors. Trop Med Int Health. 2011;16(5):598–607

92. Astrom C, Rocklov J, Hales S, Beguin A, Louis V, Sauerborn R. Potential distribution of dengue fever under scenarios of climate change and economic development. Ecohealth. 2012;9(4):448–454.

93. Australia. *Environmental health perspectives*, *120*(2), 260-266.

94. Gubler DJ, Sather GE, Kuno G, Cabral JR. Dengue 3 virus transmission in Africa. Am J Trop Med Hyg. 1986;35(6):1280–1284.

95. Murray, N. E. A., Quam, M. B., & Wilder-Smith, A. (2013). Epidemiology of dengue: past, present and future prospects. *Clinical epidemiology*, *5*, 299.

96. Kuno, G. (2009). Emergence of the severe syndrome and mortality associated with dengue and dengue-like illness: historical records (1890 to 1950) and their compatibility with current hypotheses on the shift of disease manifestation. *Clinical microbiology reviews*, *22*(2), 186-201.

97. Duong, V., Lambrechts, L., Paul, R. E., Ly, S., Lay, R. S., Long, K. C., ... & Buchy, P. (2015). Asymptomatic humans transmit dengue virus to mosquitoes. *Proceedings of the National Academy of Sciences*, *112*(47), 14688-14693.

98. Vong S, Khieu V, Glass O, et al. Dengue incidence in urban and rural Cambodia: results from population-based active fever surveillance, 2006–2008. PLoS Negl Trop Dis. 2010;4:e903

ii