Molecular and Serologic Surveillance of Dengue Infections in Jazan Region, Southwestern Saudi Arabia (2017)

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A Thesis
Submitted to the University of Gezira for Fulfillment of the Requirements for the award of the Degree of Doctor of Philosophy in Medical Microbiology

Department of Medical Microbiology
Faculty of Medical Laboratory Sciences
University of Gezira

2017
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قال تعالى

اللَّهُ نُورُ السَّمَاوَاتِ وَالأَرْضِ ۖ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ

المَصْبَاحُ فِي رُجَاجَةٍ الرُّجَاجَةُ كَانَتَا كَوَكَبٌ دُرِّيٌّ يُوقَدُ مِن شَجَرةٍ

مُبَارَكَةٍ زَيَاتُونَهُ لَا شَرِقَيَةٌ وَلَا غَربَيَةٌ يَكَادُ زَيَاتُهَا يُضِيءُ وَلَا لَمْ تَمَسْهُ

نَارٌ تُثُوبَ عَلَى نُورٍ يَهْدِي اللَّهُ لِنُورِهِ مِنْ يَسَاءٍ وَيُضْرِبُ اللَّهُ الأمَثَالَ

إِلَّا اللَّهُ عَلِيمٌ وَلَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ

صدق الله العظيم

(35) سورة النور
Dedication

My Parents, whom leads me through the valley of darkness with light of hope and support.

MY MOTHER

My wife , the special gift from Allah to me
My beloved brothers and sisters; whom stands by me when things look bleak.

To all my family, the symbol of love and giving.

To my supervisor Professor Adam Dawoud without him I couldn’t reach the success

My friends whom encourage and support me.

All the people in my life who touch my heart

I dedicate this research.
Declaration

I am Mussab Hassan Mohammed I hereby declare that the work that concern the requirements of doctor of philosophy in “Molecular and Serologic Surveillance of Dengue Infections in Jazan Region, Southwestern Saudi Arabia (2017)” at the University of Gezira, Sudan under the supervisions of Prof. Adam Dawoud Abakar Salim and Prof. Bakri Yousif Mohammed Nour, had been prepared by my own potential and it had not been copied from any other source, also it had not been presented by any other researcher for Scientific degree elsewhere.

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Acknowledgments

I want to complete my thesis with an expression of thankfulness to those who provided me with support through their knowledge, guidance, moral support and motivation to persevere in a long road to complete my thesis.

I would like to thank and appreciate the full supporting of my supervisor Prof. Adam Dawoud Abakar for his permanent support, encouragement, guidance and understanding during critical stages in the preparation of this thesis. His love for science and his gift as a mentor were a strong motivation to persevere to the end. My thanks also goes to my co supervisor Prof Bakri Yousif Mohammed Nour, for his effort during the whole period of my study.

Special thanks is due to Dr. Omer Alhassan for helping me in molecular working in this article {PCR and sequencing} and for his permanent advice, encouragement and support in different stages of my program.

My family my mother, my wife, brothers & sisters Ricardo and my friends who gave me encouragement not to give up in this sometimes steep journey to complete my degree.

Mr. Badreldin El fadil, miss Islam Abdellatif, miss Ayah Abdelaltif and to any Pearson help me I really appreciate yours help I ask Allah to bless all of them.
Molecular and Serologic Surveillance of Dengue Infections in Jazan Region, Southwestern Saudi Arabia (2017)

Mussab Hassan Mohammed Ibrahim

ABSTRACT

Dengue is a global health issue. The clinical illness ranges from an asymptomatic febrile illness to dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). The study aimed to detect any indigenous recent dengue fever virus infection in Jazan region since such infections have not been previously reported in the region, and to raise the awareness of health care workers to include the possibility of dengue virus infection when investigating febrile illnesses. A representative sample of 390 individuals attending randomly selected health care centers in the study area (Jazan Region, southwestern Saudi Arabia) were interviewed and tested for dengue virus (DENV) specific IgM against the four serotypes for evidence of recent DENV infection. Serum samples of suspected dengue cases were collected throughout November 2015 to October 2016 and tested by one step Reverse Transcription Polymerase Chain Reaction (RT-PCR) with a set of specific primers for detection of four dengue virus serotypes followed by sequencing the PCR products to confirm the results. Out of the 100 serum samples, 33 were found positive for dengue infection (33.0%). Two dengue virus serotypes were detected; DEN-2 and DEN-3. DEN-2 is the most common and predominant type in the region rating 69.7% (23/33) and then DEN-3 {30.3% (9/30)}. The present study reports, for the first time, cases of DENV infection indigenous to Jazan Region. A seroprevalence of 33 was documented. Based on this finding, health care professionals should test for DENV infection when investigating febrile illnesses so as not to miss any sporadic cases of this infection.
المسح المصلى والجزيئي للإصابة بحمى الضنك في منطقة جيزان غربي المملكة العربية السعودية (2017)

مصعب حسن إبراهيم

ملخص الدراسة

تشكل حمى الضنك مشكلة صحية عالمية وتتراوح الاعراض السريرية بين الإصابة غير البائنة المصحوبة بالحمى الى انوع النزيف ومتلازمة الضنك الصدمة. هدفت الدراسة للكشف عن الإصابة بفيروس حمي الضنك في جمنان جيزان الواقع جنوب غرب المملكة العربية السعودية واللتي لم يتم عمل بحث محكم في هذا المجال في هذا الاقليم من قبل. كما تهدف هذه الدراسة لتحسين الوعي في المجال الصحي بصورة عامة والاهتمام في وجوب الفحص في حالة الغموض عند التعامل مع المرضى الذين يعانون من الصدمة. تم اختيار 390 مريضاً عشوائياً من عدة منشآت صحية من منطقة البحث للكشف عن فيروس الضنك بانواعه الرباعية في المرضى حديث الاصابة. جمعت العينات في الفترة من نوفمبر 2015 حتى أكتوبر 2016، تم عمل تحليل السريع (ان اس 1) تحليل الاليازا وتحليل البي سي ار المختص في الكشف عن الحمض النووي للفيروس كما تم عمل كشف عن الجين للفيروسات التي تم عزلها من المرضى المصابين. من مجموع عينة وجد أن هناك 33 عينة إيجابية (33%)، تم إيجاد نوعين من فيروس حمي الضنك، فيروس حمي الضنك النوع الأول والنوع الثاني كما وجد أن معدل الاصابة بال النوع الأول هو الأكثر شيوعاً في المنطقة (69.7%) ثم النوع الثالث (30.3%). أظهرت هذه الدراسة، لأول مرة، حالات عددية ضنك أصلية في منطقة جازان وتم توثيق انتشار مصلي يبلغ 33 حالة. واستنادا إلى هذه النتائج، يجب على المهنيين الرعاية الصحية اختيار عدد من çift عند التحقيق في الأمراض الحمومية حتى لا تفوت أي حالات مفرطة من هذه العدوى.
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Chapter One
Introduction

1.1 Introduction:

Dengue (pronounced as den’gē) is the most common arboviral (arthropod-transmitted) disease and it also ranks as the most important mosquito-borne viral disease in the world. Some 2.5 billion people living in tropical and sub-tropical regions are at risk of dengue infection, which equates to about two-fifths of humanity\(^1,2\). There is an estimated 50-100 million infections occurring globally every year, with 500,000 cases requiring hospitalization and causing 24,000 deaths\(^3,4\). Furthermore, the number of people living in tropical and sub-tropical regions is set to double by the end of the century\(^5,6\), thus making dengue an unqualified global threat to public health. The term “dengue” is thought to be a Spanish homonym for the Swahili phrase “ki denga pepo”, meaning a sudden cramp-like seizure by an evil spirit or plague \(^7\). The name “break bone fever”, which is attributed to the excruciating joint pains den.

Dengue is an arthropod borne viral illness caused by one of the 4 serotypes of the Dengue virus (DEN1-4). It is worldwide in distribution especially in tropical and sub-tropical regions and is transmitted by mosquito *Aedes aegypti* (mainly) and *Aedes albopiticus* [1]. The clinical manifestations ranges from an asymptomatic febrile illness to more severe forms of infection i.e. dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) [2]. In India first outbreak of dengue fever was reported in Kolkata in 1963-64, and gradually spread to other parts of the country [3]. NVBDCP website data shows that dengue is endemic in 16 states of India and Bihar is one of them [4].

Over past two decades there has been increase in the number of Dengue cases and also a change in the trends of epidemiology is seen. Current diagnostics modalities available are detection of RNA of the virus with reverse transcriptase PCR (RT-PCR) in the serum of the patient which is a Gold standard test; viral isolation and serological tests like IgM capture ELISA (MAC ELISA) [5]. However viral isolation and RT-PCR both are time consuming, labor intensive and expensive therefore cannot be used as routine diagnostic
procedures. MAC ELISA is easy to perform so it is the most widely used serological test for the diagnosis of Dengue infection. But this test also has low sensitivity in the first week of the infection as it take 4-8 days for IgM levels to be detectable by MAC ELISA [6].

1.2 Problem identification:
Since the first report of the isolation of dengue virus (DENV) from a fatal case in 1994 of dengue fever in Jeddah[82,83]: reports addressing the demographic and the clinical characteristics, laboratory diagnosis, disease outcome and virus surveillance have been limited to Jeddah and Mecca areas where most of the dengue cases have been reported [83,84]: In 2009, the Saudi Ministry of Health reported a total of 3350 cases in the Kingdom; among those, there were only 15 cases from Jazan region. Genotyping of the local isolates has also been established [85]. In 2007, the Saudi Ministry of Health reported a case fatality rate of 4.6 per thousand (6/1308) in Jeddah [86]: A statistical index has been computed to predict the population (densities) of the vector that could be applied to predict the confirmed dengue cases [87].

1.3 Problem justification:
To our knowledge, however, no large seroprevalence surveys have been conducted in southern Saudi Arabia, where mosquito-borne infections have posed major health problems such as the outbreak of Rift Valley fever during August 2000 to September 2001 and where malaria is endemic. Furthermore, recent entomological studies have shown that adult female Aedes aegypti (the principal vector for DENV), has been detected in Jazan (where monkeys are also found and hence the potential of a sylvatic cycle for DENV), and also from various other parts of the Kingdom of Saudi Arabia and thus such regions are possible foci of dengue fever outbreaks.

1.4 Hypothesis
From the nature and environment surrounding this region we supposed that some positive cases will be found in this area.
2.1.4 Objectives

Historically surveillance may have been seen as the systematic collection of data, it is now more: data for action—in other words the ongoing systematic collection, analysis and interpretation of data, with dissemination of the resulting information to those who need to know in order that action may be taken.

2.1.4.1 General objective

The purpose of this study was to establish those epidemiological characteristics, diagnostic and clinical aspects of dengue disease that could be related with the surveillance of dengue, and use these to understand how surveillance can be used as one strategy to prevent the effect of the disease caused by dengue infection. The goal of this study is to compare and contrast the findings with the traditional or regular dengue surveillance system. Epidemiological aspects are those indicators which usually are not considered in the passive surveillance systems but are affecting the dynamic of the disease in the populations. In this study, epidemiological aspects included are frequency of dengue, silent or asymptotically infected people, and the proportion of people at risk to acquire DHF. Clinical and laboratory diagnostic aspects are those criteria which can improve the identification of the dengue cases. We considered and collected during the course of this study this information, the day of the physical exam was performed and the blood sample taken, as well as the specific time and type of diagnostic test used and the clinical symptoms presented. A surveillance system of dengue should have the capacity to collect and analyze this kind of information in order to prevent disease effects.

1.5 Specific objectives

The stated specific objectives of surveillance:

- To estimate the incidence density of dengue disease in the population, by detecting the number of symptomatic cases during active surveillance, and sorting out and analyzing data by age groups, location and seasonal year period.
- To estimate the prevalence of antibody against dengue in the population using anti-dengue IgM MAC ELISA and anti-dengue IgG sero-prevalence surveys.
• To identify and compare procedure in laboratory test used in the active surveillance, by considering viremic and immunological indicators at specific time points, which are categorized based on the number of days after onset of the symptoms, age group, neighborhoods and serotype from the active surveillance.
• To identify clinical symptoms according to disease confirmation and age group in those people who were detected in the active surveillance.
• To identify the differences between the regular system of passive surveillance of dengue disease in the national and local department of health and the data obtained in this study, assuming this study represents a system of active surveillance.
Chapter Two
Literature Review

1.1.2 Brief History of Dengue and DHF:

DENV-1 was first isolated by Ren Kimura and Susumu Hotta in Japan in 1943\(^8\). An epidemic of DF involving at least 200,000 cases had occurred between 1942 and 1944 during World War II in Japanese port cities such as Nagasaki, Kobe, and Osaka. The infections originated from persons returning from the tropics, in particular Southeast Asia and the Pacific islands\(^9\). A few months after the first isolation of DENV-1 in Japan, Albert Bruce Sabin and Walter Schlesinger isolated DENV-1 from Hawaiian and shortly thereafter, DENV-2 from Papua New Guinean samples\(^10\). They demonstrated that these viruses were antigenically related, yet distinct, and they could be distinguished by the hemagglutination inhibition (HI) assay. Although there were various speculations about the earliest description of dengue-like diseases in historical accounts\(^11,12\), the disease now known as DHF was first recognized in Manila, the Philippines in 1953\(^13\). Viruses similar to DENV-1 and DENV-2 were isolated from Manila patients in 1956 by William Hammond and were called DENV-3 and DENV-4. Dengue viruses of multiple serotypes were subsequently isolated from patients of another DHF epidemic in Bangkok, Thailand in 1958\(^14\). It is now known all four serotypes of dengue virus can cause DHF.

DHF/DSS outbreaks were mainly restricted to Southeast Asia until the early 1980s\(^11\). Since then, dengue transmission has intensified and DHF/DSS outbreaks are now frequent in most tropical countries. To this day, DHF/DSS remains a leading cause of hospitalization and death among children in Southeast Asia. Outside the region, the disease burden of dengue is most acutely felt in Central and South America where 24 countries have reported laboratory-confirmed DHF between 1981 and 1997\(^15,1,16\).
1.1.3 Symptoms
Dengue is an acute febrile viral disease caused by infection with one of the four serotypes of the dengue virus (DENV1-4). Most dengue infections are asymptomatic while the rest result in a wide spectrum of disease that differs in severity from mild undifferentiated fever, i.e. the classical dengue fever (DF), to the potentially fatal complications known as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Figure 1.1).

**Figure 1.1 Manifestations of dengue virus infection (credit: Figure 1.1 from WHO,1997)**

Common symptoms of a classic dengue fever patient include fever, fatigue, rash, headache, retro-ocular pain (pain behind the eyes), arthralgia (joint pain) and myalgia (muscle pain), nausea, vomiting and leukopenia (abnormal deficiency of leukocytes circulating in the blood). More extreme cases could include severe hemorrhage, loss of consciousness and abnormal liver and brain function. DHF, the severe form of dengue, is characterized by plasma leakage, thrombocytopenia (low platelet count) and hemorrhagic manifestations. DHF is due to increased vascular permeability believed to be caused by cytokines released when T cells attack dengue-infected cells (Halstead, 2007). The most severe form of dengue disease is DSS which includes all of the symptoms of classic dengue and DHF, with the addition of intense and
sustained abdominal pain, persistent vomiting, restlessness or lethargy, a sudden change from fever to hypothermia with sweating and prostration, and shock caused by extremely low blood pressure\(^{(17)}\).

After a patient is infected with dengue virus through the bite of an infected female mosquito, there is an incubation period that can vary between 3 and 14 days. The patient subsequently enters the painful febrile period when viremia is at its peak. Viremia ends 5-7 days after the onset of fever, coincident with defervescence. DHF/DSS usually develops around this time, and intensified observation of the patient is crucial. If DHF develops, the patient may rapidly go into a state of shock and die within 12 to 24 hours if left untreated. After effervescence, laboratory diagnosis is based on IgG and IgM antibody detection. The disease progression for dengue is presented in schematic form in (Figure 1.2).

A person could suffer from dengue infection four times throughout his/her lifetime, once for each of the four DENV serotypes. Both primary (first) and secondary (subsequent) infections with any serotype of DENV can result in either the clinically less severe DF or the more severe DHF\(^{(19)}\). A primary dengue infection confers the recovered patient lifelong immunity against the infecting serotype and a brief protection against infection by other DENV serotypes\(^{(20)}\). However, epidemiological data and some studies suggest that the immunity thus gained, after the lapse of the temporary cross-serotypic protection,

**Figure 1.2** Course of dengue infection and the timings and choices of diagnostic methods.
increases the probability of an individual developing DHF when infected by a second heterologous DENV serotype \(^{(21, 22)}\). A hypothesis to explain this phenomenon, called antibody-dependent enhancement (ADE), proposes that pre-existing sub-neutralizing antibodies from the primary infection and the second infecting DENV serotype form complexes that bind to cells bearing Fc\(\gamma\) receptor (Fc\(\gamma\)R) (monocytes and B cells) leading to increased virus uptake and replication (Figure 1.3)\(^{(3)}\).

There is no specific antiviral therapy or vaccine in clinical use for dengue fever. Medical care is supportive in nature and focuses on monitoring and administration of fluids to prevent dehydration and shock, medications to lower fever and reduce pain, and management of bleeding complications. In the late 1960s, DHF fatality has been reported to be as high as 41.3\%\(^{(23)}\) when healthcare providers understandably were still unfamiliar with the disease. Today, DHF fatality rates can exceed 20\% without proper treatment, but can be brought down to 1\% with proper medical care\(^{(4)}\).

**Figure 1.3** Model for antibody-dependent enhancement (ADE) of dengue virus replication (credit: Figure 1.3 from Whitehead et al., 2007)\(^{(27)}\).
### 1.1.4 Classification:

Although the term “dengue” is commonly used to refer to the entire spectrum of dengue disease, the WHO has devised a formal classification scheme in 1974 that defines dengue as either asymptomatic, DF or DHF/DSS\(^{(24)}\). The DHF category is further classified based on the number of hemorrhagic manifestations into four grades of severity (Table 1.1).

Grade III and IV of DHF, where profound plasma leakage occurs, are referred to as Dengue Shock Syndrome (DSS). These guidelines were developed based on pediatric cases reported to the Children’s Hospital, Bangkok, Thailand in the 1960s. In recent years, clinicians have been reporting difficulties in following these guidelines to classify the disease as dengue has spread globally with a concurrent change in patient demographic profile\(^{(25,26)}\).

**Table 1.1 WHO case definition for DHF severity.**

<table>
<thead>
<tr>
<th>Classification</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade I</td>
<td>Fever with other symptoms such as vomiting, headache, muscle and joint pain, skin rash: positive tourniquet test is the only evidence of hemorrhaging.</td>
</tr>
<tr>
<td>Grade II</td>
<td>Grade I symptoms and spontaneous bleeding.</td>
</tr>
<tr>
<td>Grade III</td>
<td>Failure of circulatory system, clammy skin, rapid and weak pulse, restlessness.</td>
</tr>
<tr>
<td>Grade IV</td>
<td>Severe shock, no measurable blood pressure or pulse.</td>
</tr>
</tbody>
</table>
1.1.5 Vectors and Transmission Cycles:

Dengue is transmitted from person to person through the bites of infected female mosquitoes. The etiological agent, the DENV, is believed to have been maintained in sylvatic/encephalotic transmission cycles involving non-human primate hosts and vector species living in forests. The virus was transmitted to humans when the two come into contact and thereafter was maintained in continuous human-mosquito cycles in and/or around human population centers (Figure 1.4).

**Figure 1.4** Transmission of dengue viruses (credit: Figure 2 from Whitehead *et al.*, 2007)

Many species from the genus *Aedes* of the family *Culicidae* are known to transmit DENV, but the principal vector is *Aedes aegypti* which is also the vector of the yellow fever virus (YFV). Moreover, this species transmits a third arboviral disease, chikungunya, which is caused by the chikungunya virus (CHIKV), an alphavirus of the family *Togaviridae*. Chikungunya has similar symptoms as dengue which often made accurate diagnosis difficult. The Australian naturalist Thomas Lane Bancroft first suggested *Ae. aegypti* as a carrier of dengue fever in 1906 based on epidemiological grounds, and this was confirmed in 1916 by John Burton Cleland(28). *Ae. aegypti* is known to be a day-biting mosquito that prefers to breed in domestic and peridomestic water containers. Its adaptation to human habitats and its desiccation-resistant eggs have allowed it to flourish in urban centers.

The secondary vector for dengue is *Aedes albopictus* which is commonly known as the
Asian tiger mosquito. Its role as dengue vector in semi-tropical regions was first identified by Koizumi et al. in Taiwan in 1917\(^{(29)}\). *Ae. albopictus* serves as the primary vector for dengue in countries where *Ae. aegypti* is absent and as a maintenance vector in rural areas where both species coexist \(^{(30,31)}\). In the Pacific islands *Ae. polynesiensis* has been suggested as the primary dengue vector\(^{(32,33)}\) whereas *Ae. scutellaris* was identified as the ‘jungle’ vector for dengue (Mackerras, 1946)\(^{(34)}\). Similar to *Ae. aegypti*, *Ae. albopictus* is also an efficient vector for CHIKV and it has been implicated in causing major chikungunya epidemics in recent years\(^{(35,36)}\).

*Ae. aegypti* and *Ae. albopictus* have both been shown to be anthropophilic, i.e. prefer to feed on humans\(^{(37)}\) and are widely distributed in both urban and semi-urban areas in the tropics and subtropics. Both species have also been demonstrated to possess high vector competence for the dengue virus\(^{(38)}\). In the continued absence of vaccines and specific treatment, effective vector control (either though fogging that kills adult mosquitoes, application of larvicides that target the aquatic stage of mosquitoes, or source reduction that reduces their breeding habitat) is currently the only practical method available for reducing the incidence of dengue disease.

1.1.6 Geographical Distribution:

DENV is the world’s most geographically widespread arthropod-borne virus and its geographical distribution is inherently tied to the range and habitat of its principal vector mosquitoes (Figure 1.5). Dengue infections are reported in more than one hundred tropical and sub-tropical countries worldwide, mostly in urban and semi-urban areas where the vectors are widely found. Dengue is hyperendemic in many of these urban centers with co-circulation of multiple dengue virus serotypes. In non-tropical regions, dengue is usually the result of infection of international travelers that have visited dengue-endemic areas.
Figure 1.5. Approximate global distribution of dengue and Aedes aegypti in 2005 (credit: Figure 1 from Halstead, 2007)(18).

The larvae of the principal vector *Ae. aegypti* under naturally changing temperature are capable of developing into adults in conditions lower than 10°C, whereas those of *Ae. albopictus* can survive even lower temperatures(39). Consequently the two species can be found between latitudes 35°N and 35°S, approximately corresponding to a winter isotherm of 10°C(4). As shown in (Figure 1.6), the southern parts of the United States and Europe, and major parts of Australia and Africa are among areas at risk of future dengue transmissions. A dengue outbreak reported in Buenos Aires, Argentina (34°36′S) in early 2009 is very close to this isotherm and is the furthest south dengue has spread.
1.1.7 Factors Influencing Transmission

Since the etiology of dengue and dengue hemorrhagic fever were virologically described in the mid-1950s, the incidence of dengue worldwide has increased tremendously (Figure 1.7). There is a plethora of inter-related factors that contributed to the prevalence of dengue around the globe. For vector-borne diseases such as dengue, these factors can be categorized into three obvious components – virus, vector and host – and a less-clear fourth – the surrounding ecology for the three components. All four play important roles in the continued spread and transmission of dengue. Uncontrolled urbanization, expanding urban population, poverty, ineffective public health infrastructure, faster modes of transportation, globalization of trade and increased international travel have all been implicated as factors leading to the spread of dengue around the world\(^1\). Rapid urbanization is probably the single most important contributing factor – the resulting population centers tend to lack public piped water and residents have to resort to using containers to store water which often ended up as breeding sites for the *Ae. aegypti* vector. The lack of adequate sewage systems often leads to the same result.

Inherent differences in the virulence of the introduced DENV strains have also been
suggested as being a contributing factor in causing outbreaks and in the emergence of the severe form of dengue disease\textsuperscript{(19)}. An often-cited example is the replacement of the indigenous American genotype of DENV-2 in the Western Hemisphere with one originating from Southeast Asia \textsuperscript{(40)}. Viruses of the Southeast Asian genotype have been shown in the laboratory to be better adapted to transmission by the vector \textit{Ae. aegypti} by causing higher viremia in both human dendritic cells and mosquito cells\textsuperscript{(41,42,43)}.

Relaxation of vector control efforts, expansion of the vector range, and the build-up of vector resistance to insecticides (Gubler and Clark, 1995\textsuperscript{(1)}; Kawada et al., 2009\textsuperscript{(44)}) are some of the recognized factors affecting the contribution of the mosquito vector. The impact of environmental factors on the transmission and spread of mosquito-borne diseases - as exemplified by effects of temperature, rainfall and humidity on vector transmission cycles - are also well known\textsuperscript{(45)}.

\textbf{Figure 1.7 Average annual number of DF/DHF since 1955}

Beside the effect of generalized climatic factors (global warming, for example) the local ecology probably plays an equal, if not more important, role in a disease as complex as dengue\textsuperscript{(46,47,48)}. 

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1.1.8 Economic impact:

Apart from physical pain, dengue also causes economic hardship to recovered individuals in the form of hospitalization costs and disruption of earning potential. The DALYs (disability-adjusted life years) lost to dengue in Southeast Asia were estimated to be comparable to the burden caused by diseases such as HIV, malaria and tuberculosis\(^4\). At the governmental level, vast amounts of money have to be allocated for public awareness campaigns, medical services and vector eradication efforts. Another indirect cost comes in the form of loss of revenue through reduced tourism\(^4\).

1.2 Dengue the Virus:

The causative agent of the dengue disease is the dengue virus (DENV), a group of four flaviviruses that are closely related but antigenically distinct. They are hypothesized to have evolved independently from ancestral sylvatic viruses between 100-1,500 years ago\(^5\). The four groups are known as serotypes and denoted as dengue virus type 1 (DENV-1), dengue virus type 2 (DENV-2), dengue virus type 3 (DENV-3) and dengue virus type 4 (DENV-4).

1.2.1 Taxonomy:

There are three genera in the Flaviviridae family (formerly known as group B arboviruses) namely Flavivirus, Pestivirus and Hepacivirus. The dengue virus is a member of the genus Flavivirus which consists of 55 identified virus species\(^5\). The word Flavi is a derivation from the Latin “flavus” which means “yellow” and the type species of the genus is the yellow fever virus (YFV). The flaviviruses are thus named due to the jaundice observed in yellow fever patients. Many flaviviruses are important human pathogens, most notably the dengue viruses, yellow fever virus, Japanese encephalitis virus (JEV), West Nile virus (WNV) and tick-borne encephalitis virus (TBEV). The flaviviruses are predominantly transmitted by mosquitoes and ticks, whereas some have no known vector.

Dengue was one of the groups classified when early researchers divided the flaviviruses serologically into eight antigenic complexes using cross-neutralization tests. However,
many viruses, for example the prototype of the genus YFV, could not be affiliated with any complexes\(^{(52)}\). When sequence data became available, phylogenetic inference from molecular data showed agreement with the antigenic complex classification. In addition, it revealed the clear clustering of the *Flavivirus* genus into non-vector and vector-borne virus clusters, with the latter splitting into mosquito-borne and tick-borne virus clusters\(^{(53)}\). As shown in (Figure 1.8), the mosquito-borne virus cluster has been shown to further diverge into YFV, JEV, and dengue viruses, in that order\(^{(54)}\).

The dengue virus was divided into four groups called *serotypes* based on antigenic properties. Subsequent evidence from molecular data reaffirmed this classification and also provided a clearer understanding of the phylogeny of the four serotypes: among the dengue viruses, DENV-4 diverged first from the common ancestor, followed by DENV-2, and finally DENV-1 and DENV-3 \(^{(54)}\).

### 1.2.2 Virion Morphology:

The dengue virus virion, like those of other flaviviruses, is spherical and 40-50 nm in diameter. It is comprised of a nucleocapsid about 30 nm in diameter that is enclosed in a lipid envelope. The nucleocapsid contains the viral capsid and RNA genome. The lipid-containing envelope consists of a lipid bilayer, an envelope protein between 51,000 and 59,000 Daltons that mediates attachment, fusion, and penetration, and a small non-glycosylated internal matrix protein of approximately 8,500 Daltons. The envelope protein is glycosylated in most flaviviruses and is exposed on the virion surface.
Electron microscopy studies have shown that mature dengue virions are characterized by a relatively smooth surface, as shown in (Figure 1.9), with 180 copies of the envelope protein forming the icosahedral scaffold\(^{(56)}\).

**1.2.3 Genomic Organization:**

The genomic organization of the dengue virus, and by extension all flaviviruses, is relatively simple compared to other arboviral families such as the *Togaviridae* (formerly known as group A arboviruses), *Bunyaviridae* or *Rhabdoviridae*. The DENV genome consists of a single-stranded, positive-sense RNA molecule roughly 10.7 kb in size.
Figure 1.9 Structure of the dengue virion and conformations of the E protein (credit: Figure 2 from Perera and Kuhn, 2008)\textsuperscript{(55)}. ER: endoplasmic reticulum; TGN: trans-Golgi network; prM: precursor of membrane.

It contains a single translated open reading frame (ORF) that encodes a precursor polypeptide of around 3390 amino acids which is processed catalytically into ten viral proteins (Table 1.2).
Table 1.2. Typical lengths of the ten DENV proteins determined from multiple sequence alignments of deduced amino acid sequences derived from complete genome sequences in GenBank.

<table>
<thead>
<tr>
<th>Proteins</th>
<th>DENV-1</th>
<th>DENV-2</th>
<th>DENV-3</th>
<th>DENV-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>114</td>
<td>114</td>
<td>113</td>
<td>113</td>
</tr>
<tr>
<td>prM/M</td>
<td>166</td>
<td>166</td>
<td>166</td>
<td>166</td>
</tr>
<tr>
<td>E</td>
<td>495</td>
<td>495</td>
<td>493</td>
<td>495</td>
</tr>
<tr>
<td>NS1</td>
<td>352</td>
<td>352</td>
<td>352</td>
<td>352</td>
</tr>
<tr>
<td>NS2A</td>
<td>218</td>
<td>218</td>
<td>218</td>
<td>218</td>
</tr>
<tr>
<td>NS2B</td>
<td>130</td>
<td>130</td>
<td>130</td>
<td>130</td>
</tr>
<tr>
<td>NS3</td>
<td>619</td>
<td>618</td>
<td>619</td>
<td>618</td>
</tr>
<tr>
<td>NS4A</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>NS4B</td>
<td>249</td>
<td>248</td>
<td>248</td>
<td>245</td>
</tr>
<tr>
<td>NS5</td>
<td>899</td>
<td>900</td>
<td>900</td>
<td>900</td>
</tr>
<tr>
<td>Length of CDS</td>
<td>3392</td>
<td>3391</td>
<td>3390</td>
<td>3387</td>
</tr>
</tbody>
</table>

There is no evidence of alternative or overlapping reading frames that are translated and there is also no hyper-variable region in the DENV genome like those reported in the
HCV genome.
The DENV ORF is flanked at its 5’ terminus by an untranslated region (UTR) of about 100 nucleotides and a longer UTR of about 500 nucleotides at its 3’ terminus. The 5’ terminus of the genome has a type I cap (m\(^7\)GpppAmp) and there is no polyadenylation of the 3’ terminus\(^{(57)}\).

The translated polyprotein is cleaved co- and post-translationally by viral and host proteases into ten viral proteins: three structural proteins (C, capsid; prM/M, precursor of membrane; E, envelope) the 5’ end of the ORF, and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) encoded at the 3’ end (Figure 1.10).

**Figure 1.10.** Schematic diagram showing: (top) gene organization in the dengue virus RNA genome, (bottom) the membrane topology and proteolytic cleavage sites of the transcribed polyprotein. Cellular and viral proteases, which are denoted by arrows, process the immature polyprotein into ten separate proteins (credit: adaptation of Figure 1 from Perera and Kuhn, 2008)\(^{(55)}\).
The three structural proteins constitute the DENV virion: the capsid protein surrounds the viral RNA genome to form the nucleocapsid, whereas the prM and E proteins are embedded in the lipid bilayer that forms the viral envelope. Cleavage of the prM into the membrane (M) protein by furin during viral release has been shown to be a prerequisite for the production of mature infectious virions. Of the three structural proteins, the E protein is the most studied as it is the major constituent of the virus envelope. It is glycosylated at two sites (Asn-67 and Asn-153) and is responsible for virus attachment to receptors of susceptible host cells and for fusion with cell membranes.

The E glycoprotein also contains the main epitopes recognized by neutralizing antibodies\(^\text{(57)}\). Such epitopes are also found to a lesser extent on the M glycoprotein\(^\text{(58)}\). The 3’ end of the DENV genome encodes seven non-structural (NS) proteins of various sizes in the order: NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. Some non-structural proteins are known to be multi-functional while little is known about NS1, NS2A and NS4A/4B. Functions of the non-structural proteins are summarized in (Table 1.3).
Table 1.3 Known and possible functions of dengue non-structural proteins (reviewed in Perera and Kuhn, 2008)\(^{(55)}\).

<table>
<thead>
<tr>
<th>NS proteins</th>
<th>Description of known functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS1</td>
<td>Plays a role in viral RNA replication complex; acts as soluble complement-fixing antigen.</td>
</tr>
<tr>
<td>NS2A</td>
<td>Forms part of the RNA replication complex.</td>
</tr>
<tr>
<td>NS2B</td>
<td>Co-factor for NS3 protease.</td>
</tr>
<tr>
<td>NS3</td>
<td>Serine protease, RNA helicase and RTPase/NTPase.</td>
</tr>
<tr>
<td>NS4A</td>
<td>Possibly induces membrane alterations important for virus replication.</td>
</tr>
<tr>
<td>NS4B</td>
<td>Possibly blocks IFN α/β-induced signal transduction.</td>
</tr>
<tr>
<td>NS5</td>
<td>Methyltransferase (MTase) and RNA-dependent RNA polymerase (RdRp).</td>
</tr>
</tbody>
</table>

1.2.4 Genetic Diversity:

Based on available molecular data it is well known that there is great genetic diversity among the dengue viruses. The factors that contributed to this are many fold, so are the
epidemiological implications arising from this diversity, as illustrated in (Figure 1.11).
For most purposes the four dengue serotypes are generally treated as the same virus and the diseases they cause are considered as the same disease.

**Figure 1.11** The processes that have caused an increase in the genetic diversity of dengue virus and two possible evolutionary consequences of this increase (credit: Figure 1 from Holmes and Burch, 2000)(59).

However, the genetic distances between the four serotypes are greater than the distances between many of the recognized virus species in the genus, for example between the Japanese Encephalitis virus (JEV), West Nile virus (WNV), Murray Valley Encephalitis virus (MVEV), Usutu virus (USUV) and St Louis Encephalitis virus (SLEV) (Figure 1.12). Based on this observation, others have argued that the four dengue serotypes warrant the rank of species on their own right(53,59).

Each serotype of the dengue virus can be further classified into several genetic groups called genotypes (the term subtype is used interchangeably) based on sequence diversity. Rico-Hesse (1990)(60) initially defined a dengue genotype as a group of dengue viruses having no more than 6% sequence divergence within a 240-nucleotide region of the DENV-1 and DENV-2 E/NS1 junction.
Since then, both the length and region of virus genome selected for sequencing varied greatly depending on research groups, ranging from the complete sequence of single genes to the complete genome of the DENV. Assignment of genotypes now relies on phylogenetic analysis rather than arbitrary cut-off values in sequence diversity. (Rico-Hesse, 2003\(^{(61)}\); Vasilakis and Weaver, 2008\(^{(62)}\)) have published excellent and detailed descriptions of the genotype classification for all four dengue serotypes. The following paragraphs describe only the essential points of the subject matter. DENV-1 can be divided into five genotypes based on the complete E gene sequence as described by (Goncalvez et al., 2002)\(^{(63)}\). Earlier work by (Rico-Hesse, 1990)\(^{(60)}\) also classified DENV-1 into five groups based on the 240-nucleotide E/NS1 junction sequences, but with some minor differences from the newer scheme which is listed in (Table 1.4).

The DENV-1 genotypes all have a wide area of distribution apart from genotype III (sylvatic) and genotype II which consists of Thai strains from the 1950s and 1960s. Viruses of genotype I and IV have recently been implicated as causing epidemics in the Pacific between 2000 and 2004\(^{(64)}\) and genotype V viruses are frequently isolated during epidemics in the Americas\(^{(65)}\). However, it is still inconclusive whether any of these three DENV-1 genotypes can be consistently associated with causing more severe dengue\(^{(61)}\).
Table 1.4 DENV-1 genotypes according to Goncalvez et al. (2002)

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Original known distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Japan, Hawaii in the 1940s (the prototype strains), China, Taiwan and Southeast Asia.</td>
</tr>
<tr>
<td>II</td>
<td>Thailand in the 1950s and 1960s.</td>
</tr>
<tr>
<td>III</td>
<td>Sylvatic source in Malaysia.</td>
</tr>
<tr>
<td>IV</td>
<td>Nauru, Australia, Indonesia and the Philippines.</td>
</tr>
<tr>
<td>V</td>
<td>Africa, Southeast Asia and the Americas.</td>
</tr>
</tbody>
</table>

DENV-2 is the most studied serotype among the dengue viruses. (Twiddy et al., 2002)\textsuperscript{(66)} proposed the existence of six genotypes of DENV2 (Table 1.5) based on the complete E gene sequence following earlier work by (Rico-Hesse, 1990)\textsuperscript{(60)}, Lewis et al., 1993\textsuperscript{(67)}). Sylvatic DENV-2 strains that are closely related have been isolated from several countries in West Africa and Malaysia, two locations that are far apart\textsuperscript{(50)}, leading to hypothesis that the DENV sylvatic ancestor arose in the Asian-Oceanic region before diverging into today’s four DENV serotypes. The first DHF epidemic in the Americas occurred after an introduction of the Asian II genotype to Cuba in 1981\textsuperscript{(68)}. Likewise, the America/Asian genotype (genotype III) has been reported to have replaced the pre-existing American genotype (genotype V) in the Western Hemisphere\textsuperscript{(40)} and is considered to be the DENV-2 genotype with the highest epidemiological impact\textsuperscript{(61)}. 
Table 1.5 DENV-2 genotypes according to Twiddy *et al.* (2002)\(^{(66)}\)

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Original known distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>American</td>
<td>Formerly known as subtype V. Found in Latin America, old strains from India (1957), the Caribbean, and the Pacific islands between 1950 and 1970s.</td>
</tr>
<tr>
<td>American/Asian</td>
<td>Formerly known as subtype III. Found in China, Vietnam, Thailand and in Latin America since the 1980s.</td>
</tr>
<tr>
<td>Asian I</td>
<td>Thailand, Myanmar and Malaysia.</td>
</tr>
<tr>
<td>Asian II</td>
<td>Formerly known as subtype I and II. Found in China, the Philippines, Sri Lanka, Taiwan and Vietnam. Includes the New Guinea C prototype strain.</td>
</tr>
<tr>
<td>Cosmopolitan</td>
<td>Formerly known as genotype IV. Wide distribution including Australia, the Pacific islands, Southeast Asia, the Indian subcontinent, Indian Ocean islands, Middle East, and both East and West Africa.</td>
</tr>
<tr>
<td>Sylvatic</td>
<td>Isolated from non-human primates in West Africa and Malaysia.</td>
</tr>
</tbody>
</table>

The current genotype classification for DENV-3 follows the nomenclature proposed by (Lanciotti *et al.*, 1994)\(^{(69)}\) which recognized four DENV-3 genotypes based on prM/E sequences (Table 1.6). These four genotypes are similar to the four groups described by
(Chungue et al., 1993)\(^{(70)}\) using a 195-nucleotide region at the 5’ terminus of the E gene. Introduced to the Americas via Nicaragua in 1994, genotype III DENV-3 is now widely found in Central and Southern America\(^{(71,72,73)}\) and is considered as the most virulent of the four DENV-3 genotypes. It is worthy of note that genotype IV has never been associated with any DHF epidemics\(^{(69)}\). Although their existence is anticipated through the presence of DENV-3 antibodies in non-human canopy-dwelling primates, no sylvatic lineage of DENV-3 has been found thus far\(^{(74)}\).

**Table 1.6.** DENV-3 genotypes according to Lanciotti et al. (1994)\(^{(69)}\) and the known distribution of the genotypes prior to 1993.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Original known distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Indonesia, Malaysia, Thailand, Burma, Vietnam, the Philippines and the South Pacific islands (French Polynesia, Fiji and New Caledonia). Includes the H87 prototype strain.</td>
</tr>
<tr>
<td>II</td>
<td>Thailand, Vietnam and Bangladesh.</td>
</tr>
<tr>
<td>III</td>
<td>Singapore, Indonesia, South Pacific islands, Sri Lanka, India, Africa and Samoa.</td>
</tr>
<tr>
<td>IV</td>
<td>Puerto Rico and French Polynesia (Tahiti).</td>
</tr>
</tbody>
</table>

(Lanciotti et al., 1997) initially separated DENV-4 into two genotypes, I and II, based on the complete E gene sequence. A further two genotypes were subsequently described (Table 1.7), with one found only in non-human primates in Malaysia and another, genotype III, found only in Bangkok, Thailand\(^{(75)}\). Genotype II DENV-4 is the most widespread of the four following an introduction to the Western hemisphere in 1981,
possibly via the Pacific islands (76,77). Although DENV-4 is the least frequently sampled serotype, it is often associated with hemorrhagic fever during secondary infection (78).

Table 1.7. DENV-4 genotypes and their known distribution.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Original known distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Thailand, Malaysia, the Philippines and Sri Lanka. Includes the H241 prototype strain.</td>
</tr>
<tr>
<td>II</td>
<td>Indonesia, Malaysia, Tahiti, the Caribbean islands (Puerto Rico and Dominica) and the Americas.</td>
</tr>
<tr>
<td>III</td>
<td>Thailand (Bangkok, specifically).</td>
</tr>
<tr>
<td>Sylvatic</td>
<td>Isolated from non-human primates in Malaysia.</td>
</tr>
</tbody>
</table>

1.2.5 Role of virus genetics and evolution:

Except for the sylvatic genotypes, genotype classification can often unveil the geographical origin of the dengue virus strains. This has enabled tracking the route of virus transmissions across distant time and place, and has served as the basis of molecular epidemiological studies that can determine whether dengue epidemics are caused by introduction of new viruses or the result of re-emergence of endemic strains. Introduction of new viruses inevitably leads to the question whether particular genotypes of DENV are associated with higher virulence or severe disease. To date, severe disease has often been associated with several DENV genotypes originating in Southeast Asia (68,40). The lack of a suitable animal model for the dengue disease, however, means such hypotheses cannot be easily verified (61). On the other hand, association of re-emergence of endemic strains
with outbreaks leads to a different question that can only be answered by a combination of classic epidemiology and comparative genomics: whether the viruses re-emerged due to environmental, population immunity and/or vectorial factors, or whether outbreaks were triggered by adaptive evolution of the virus that endowed it with an increase in fitness and virulence?.

Possession of the complete genetic blueprint of the dengue viruses is a prerequisite to answering these crucial questions. This thesis describes efforts to sequence and then examine the complete genome sequences of DENV isolates from two recent epidemics in Indonesia and Singapore. The results from these two studies showed that the re-emergence of endemic strains is likely to be the main cause of most dengue outbreaks in Southeast Asian countries. No evidence of mutational signatures that could serve as a trigger of epidemics was found in isolates collected up to six years prior to the epidemic. Lastly, a new way of reconstructing the history of dengue virus diversity from all existing sequence data was introduced. The result showed that Malaysia, a country occupying a central position in Southeast Asia, has experienced both frequent importations of DENV strains from neighboring countries and maintenance of endemic viral lineages which have been in sustained transmission for many decades.

1.2.6 Vaccination:

Due to the current inability to reduce dengue transmission through vector control, a vaccine is needed to prevent transmission and disease in endemic areas\(^{(79)}\). Currently there is no licensed dengue vaccine for use in humans. Adaptive immunity contributes to resolution of infection and has a role in preventing reinfection. Unfortunately, adaptive immunity also plays an important role in the enhancement of disease severity as discussed above\(^{(27)}\). Therefore, immunization against dengue must address issues of protective immunity and the role of pathogenic immunity\(^{(27)}\).

There are many challenges to the development of an effective dengue vaccine\(^{(80,27)}\). Currently, we do not entirely understand immunity to DENV and the correlates of protective immunity are unknown. Due to the risk of more severe disease with a heterologous secondary infection, an effective vaccine must induce protective immunity
against all four serotypes of DENV\textsuperscript{(81)}. In endemic areas, a dengue vaccine must be able to overcome pre-existing immunity from either passively transferred maternal antibodies or a previous dengue infection\textsuperscript{(27)}. Additionally, an effective vaccine must induce long-term humeral and cell-mediated immunity\textsuperscript{(80)}. Thus, the ideal dengue vaccine would meet the following criteria reviewed in\textsuperscript{(27,80)}: be free from significant reactogenicity, induce lifelong immunity against infection by any of the four dengue serotypes, be suitable for use in infants, not increase the risk of DHF/DSS from concomitant or subsequent dengue exposure, induce long-term humoral and cell-mediated immunity, and be economical to produce with minimal or no repeat immunizations. Current efforts to develop a dengue vaccine can be divided into four groups: live attenuated virus vaccines, chimeric vaccines, inactivated virus vaccines, and subunit/vectored vaccines.
Chapter Three
Materials and Methods

2.2.1 The study area:

Jazan Region in Southwest Saudi Arabia lies between 16°-12, and 18°-25, latitude north. It is bordered in the South by Arabic republic of Yemen (Fig.2.1) with total area of about 22,000 km² and 1.3 million populations (The Saudi Ministry of Health, 2010). Thirty percent of the population concentrated in six major cities, and the remainders living in over 3500 villages. Jazan region is situated in the subtropical zone and has average monthly temperatures ranging between 25.8°C in January to 33.4°C in July. The average relative humidity ranges between 55% and 72.5%. The rainy season is started at August through October with a monthly average of 77 and 56.7 mm, respectively. Jazan is divided into eleven small provinces (Al-Aridah, Damad, Twal, Al-Ahad, Jazan , Al-Khobah, Samttah, Abuareesh, Sabyah, Beash and Al-Darb), these locations although with different altitudes and geographical Characteristics, they are almost share the same demographical, agricultural, educational, cultural, housing, health system, and environmental characteristics.

2.2.2 Case definition:

DF and DHF were diagnosed according to 1997 World Health Organization (WHO) classification criteria and was applied to each case after review of study notes. The 1997 definitions were used for this study because at the time of clinical assessment the 2009 WHO Guidelines and revised classification scheme was not available.
Figure 2.1 Map of the Middle East showing the study region (Jizan) in Saudi Arabia

DF was defined as a laboratory confirmed dengue case with no evidence of capillary permeability as defined for a DHF case. DHF was defined as laboratory confirmed dengue case with thrombocytopenia (<100,000 platelets/mm³), any hemorrhagic manifestation, and evidence of plasma leakage (as denoted by a >20% increase in the Hct from the baseline value or by the presence of pleural or abdominal effusions).

2.2.3 Sample size

A cross-sectional (prevalence) study was conducted from November 2015 to May 2017 in hospitals of randomly spotted areas (Figure 2.2) in Jazan region Kingdom of Saudi Arabia. A total of 390 hospitalized and non-hospitalized cases suspected of having Dengue fever, with non-specific fever, coupled with two or more of the following: headache, retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic manifestations, and leucopenia with no localized signs or symptoms were examined clinically and blood samples were collected when suspected cases were first seen. Sample size was calculated by using the WHO Manual for Sample Size Determination in Health Studies(89): with a conservative anticipated population proportion of 21% (the actual prevalence figure in a similar study in Jeddah, Saudi Arabia)(83) and with an absolute precision of 3% at 95% CI.
2.2.4 Sampling procedure:

Areas with similar socio-economic / environmental conditions and of a similar low altitude were randomly selected. Relevant district hospitals were contacted and informed consents were obtained from each individual willing to participate in the study. In case of children, consents were obtained from their guardians. Thus, a random sample of patients attending the outpatients’ clinics of these hospitals for any reasons was included in the study.

2.2.5 Ethical consideration:

The confidentiality of all participants will be maintained throughout the study. All forms with identifiers will be maintained in a limited access office. In the reporting of the laboratory results, names were used, but the information was only provided to the head of the household or the attending physician. All demographic, clinical, epidemiological, and laboratory data on each sample were entered into a database by the unique identification number. The risks of infection with venipuncture sampling were minimized by using only trained personnel to perform the venipuncture procedures using sterile, single use needles, alcohol/betadine wipes and bandages. All official protocol files (protocol, IRB minutes, and approvals) were maintained under password protection.

**Figure 2.2** Map of jazan region with details showing the study areas
2.2.5 Data collection:
A comprehensive questionnaire interview was offered to all participants, specially designed forma with three sections will use for data collection. Section A of the Performa included demographic details including age, gender, ethnicity, residence, occupation and recent travel outside the Kingdom. Section B inquired about details of clinical profile and examination. Section C recorded information on various parameters of blood tests.

2.2.6 Blood sampling:
About 5-10ml venous blood samples from adults or a maximum of 5ml from children in plain tubes were taken from each participant and were allowed to clot at room temperature (range 18ºC to 20ºC). Samples were then centrifuged at 10,000 rpm for 10min. and the separated sera were alliquotted into 2 portions and stored at –20ºC until transported in ice boxes to the Virus Lab of Jazan university College of Medicine, where they were grouped by the area of collection and stored in classified boxes in similar conditions as described above.

2.2.7 laboratory analysis:
Sera from all suspected cases were tested in the Laboratory

2.2.7.1 Non-structural protein 1 (NS1):
Detection of the DV nonstructural protein 1 (NS1) has emerged as an alternative biomarker to both serologic and molecular based techniques for diagnosis of acute DV infection. NS1 antigenemia is detectable within 24 hours and up to 9 days following symptoms onset. This overlaps with the DV viremic phase and NS1 is often detectable prior to IgM seroconversion. Concurrent evaluation for the NS1 antigen alongside testing for IgM- and IgG-class antibodies to DV (DENGM) provides optimal diagnostic potential for both early and late dengue disease.

2.2.7.2 Enzyme-Linked Immunosorbent Assay (ELISA) :
All suspected cases were tested for anti-dengue immunoglobulin (IgM and IgG) by Enzyme Linked Immunoassay (ELISA). Patients were confirm to have DF or DHF, if IgM alone or both IgM and IgG positive, these patients were prospectively follow for clinical and laboratory profile.
2.2.7.3 RNA isolation:

High Pure Viral Nucleic Acid Kit from Roche applied science (Germany) used for extraction of RNA follow the manufacture procedure; 200 μl of binding buffer supplemented with poly (A) and 50 μl Proteinase K added to 200 μl of serum sample then mixed immediately and incubated for 10 minutes at 72°C. Addition of 100 μl Proteinase K was mixed with sample and transferred to High Filter Tube inserted into Collection Tube. After centrifugation for 1 minute at 10000 rpm, the collection tube was discarded. The filter tube combined with new collection tube and 500 μl of inhibitor removal buffer was added and centrifuged for 1 minute at 10000 rpm. After changing collection tube, the high filter tube washed twice by adding 450 μl of wash buffer at the same condition of centrifugation, followed by centrifugation for 15 seconds at 13000 rpm to remove any residual wash buffer. Then the high filter tube was inserted into nuclease free, sterile 1.5 ml centrifuge tube and 50 μl of elution buffer was added to elute the viral nucleic acid by centrifugation at 10000 rpm for 1 minute.

2.2.7.4 Reverse Transcriptase Polymerase Chain Reaction (RT-PCR):

One step RT-PCR is a rapid, sensitive, and simple for dengue serotype-specific diagnosis method. The test was performed according to the protocol of Lanciotti et al (1992) with some modification; DEN consensus primers and serotype-specific primers (Table 2.2) were used to amplify the viral genome in this study and synthesized in Integrated DNA Technology (Belgium). The one step RT-PCR reactions were performed according to access RT-PCR–system protocol (Promega-USA) in total vol-ume of 50 μl containing 10 μl of AMV/Tfl 5X Reaction Buffer, 1 μl of dNTP Mix (10mM each dNTP, final con-centration 0.2mM), 2 μl of 25mM MgSO4 (final concentration 1mM), 1 μl of AMV Reverse Transcriptase 5u/ μl (final concentration 0.1u/μl), 1 μl of Tfl DNA Polymerase 5u/μl (final concentration 0.1u/μl), 50pmol (final concentration 1μM) of each forward (D1) and reverse (D2) primers, 5 μl of RNA virus and nuclease free water to total volume 50 μl. The thermal cycling incubations temperatures programmed as follows: incubation for 1 hour at 42°C (to convert the RNA to cDNA) then initial denaturation for 3 minutes at 94°C followed by 35 cycle of denaturation (94°C, for 30 second), primers annealing (55°C for 1 minute), primer extension (72°C for 2 minutes) and final extension for 5 minutes.
Table 2.1 Oligonucleotide primers used in RT-PCR and Nested-PCR

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence 5’ – 3’</th>
<th>Genome position</th>
<th>Size in bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>TCAATATGCTGAAACGCGCGAGAAACCG</td>
<td>134-161</td>
<td>511</td>
</tr>
<tr>
<td>D2</td>
<td>TTGCACCAACAGTCAATGTCTTCAGGTTTC</td>
<td>616-644</td>
<td>511</td>
</tr>
<tr>
<td>TS1</td>
<td>CGTCTCAGTGATCCGGGGG</td>
<td>568-586</td>
<td>482 (D1 and TS1)</td>
</tr>
<tr>
<td>TS2</td>
<td>CGCCACAAGGGCCATGAACAG</td>
<td>232-252</td>
<td>119 (D1 and TS2)</td>
</tr>
<tr>
<td>TS3</td>
<td>TAACATCATCATGAGACAGAGC</td>
<td>400-421</td>
<td>290 (D1 and TS3)</td>
</tr>
<tr>
<td>TS4</td>
<td>CTCTGTTGTCTTTAAACAAGAGA</td>
<td>506-527</td>
<td>392 (D1 and TS4)</td>
</tr>
</tbody>
</table>

2.2.7.5 Nested-PCR:

Nested PCR was performed in 2 tubes for each sample in 50 μl reaction mixture containing 25 μl GoTag®G2 green master mix ready to use from Promega, 10 μl of the diluted (1:100) RT-PCR product, 50 pmol (final concentration 1μM) of each forward primer D1 and TS1, TS3 as reverse primers for the first tube and TS2, TS4 as reverse primers for another tube. The samples were subjected to initial denaturation at 94°C for 3 minutes, 30 cycles of denaturation (94°C, 30 s), primer annealing (55°C, 1 min), primer extension (72°C, 2 min) and final extension for 5 minutes. In each run negative and positive controls were included. The PCR products of nested amplification were analyzed by gel electrophoresis (1.5 agarose in Tris-Acetate EDTA buffer) staining with ethidium bromide. The visualization was carried out using Gel Doc XR Imaging System (Bio-Rad).

2.2.7.6 Sequencing and bioinformatics analysis:

Purification and standard sequencing for RT-PCR products were performed by Macrogen Company (Seoul, Korea). Sequencing reactions were performed in a MJ Research PTC-225 Peltier Thermal Cycler using a ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems), following the protocols supplied by the manufacturer. Single-pass sequencing was performed on each template using D1 (forward) primer. The fluorescent-labeled fragments were purified from the unincorporated
terminators with Big Dye®X Terminator™ purification protocol. The samples were resuspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Applied Biosystems). The sequences were searched for sequence similarity through BLAST (www.ncbi.nlm.nih.gov/BLAST/) (Atschul et al., 1997) and compared to reference sequences of Dengue sero-types detected in BLAST and downloaded from GenBank (www.ncbi.nlm.nih.gov/genbank/).

Similarity tree was obtained from database online by phylogeny.fr (http://www.phylogeny.fr/)
Chapter Four

Results

Participants with aged ranged between 9 to 92 years old were recruited in longitudinal study. Enrolled people were selected during visiting outpatient clinics and hospitals with fever in thirteen different cities, neighborhoods and villages of Jazan region, southwestern Saudi Arabia. The areas selected were: (Al-Aridah, Damad, Al-Ahad, Jazan city, Al-Reath, Samttah, Abuareesh, Sabyah, Beash, Al-Edabi, Bani malek, Faifa and Al-Darb), thirty samples were collected from each of thirteen selected areas. The goals were to detect acute dengue cases in an active surveillance of fever and dengue symptoms, and to identify antibodies anti-dengue virus in blood samples.

A total of 390 patients were reported as suspected cases of dengue fever with blood samples collected from November 2015 to ending September 2017, out of which 186 were confirmed to be positive serologically by using NS1 Immunochromatography test for dengue infection amounting to 47.69% positivity rate. All these positive results were tabulated for analysis purpose. Of the 186 dengue NS1 positive sera 58(31.19%) were positive by NS1+IgM+IgG antigen, 100(53.76%) by both NS1+IgM and 28(15.05%) by NS1+IgG respectively (Table 3.1).

Table 3.1. Results of positive cases as per serological marker among 186 positive results

<table>
<thead>
<tr>
<th>Markers</th>
<th>No. of samples positive</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS1</td>
<td>186</td>
<td>100.00</td>
</tr>
<tr>
<td>NS1 +IgM</td>
<td>100</td>
<td>53.76</td>
</tr>
<tr>
<td>NS1 +IgG</td>
<td>28</td>
<td>15.05</td>
</tr>
<tr>
<td>NS1+IgM+IgG</td>
<td>58</td>
<td>31.19</td>
</tr>
</tbody>
</table>

The highest number of cases were recorded in the month of December 32(17.20%) followed by January 22(11.83%) and February 21(11.29%), but lowest number was recorded in the month of May & June 9(4.84%) (Table 3.2).Positive cases among 13 selected areas was between the highest in sabya 22 cases (11.83%) and Bani malek 21(11.29%) to the least in Al-Darb and jazan city 9(4.84%) (Table 3.3).

Table 3.2. Depicts month wise distribution of positive cases among 186 positive results
<table>
<thead>
<tr>
<th>Month</th>
<th>No. of samples positive</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>22</td>
<td>11.83</td>
</tr>
<tr>
<td>February</td>
<td>21</td>
<td>11.29</td>
</tr>
<tr>
<td>March</td>
<td>15</td>
<td>8.07</td>
</tr>
<tr>
<td>April</td>
<td>11</td>
<td>5.91</td>
</tr>
<tr>
<td>May</td>
<td>9</td>
<td>4.84</td>
</tr>
<tr>
<td>June</td>
<td>9</td>
<td>4.84</td>
</tr>
<tr>
<td>July</td>
<td>12</td>
<td>6.45</td>
</tr>
<tr>
<td>August</td>
<td>11</td>
<td>5.91</td>
</tr>
<tr>
<td>September</td>
<td>13</td>
<td>6.99</td>
</tr>
<tr>
<td>October</td>
<td>14</td>
<td>7.53</td>
</tr>
<tr>
<td>November</td>
<td>17</td>
<td>9.14</td>
</tr>
<tr>
<td>December</td>
<td>32</td>
<td>17.20</td>
</tr>
</tbody>
</table>

P < 0.05

**Table 3.3.** Positive results among areas with percentages

<table>
<thead>
<tr>
<th>Area</th>
<th>Number of positive cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabyah</td>
<td>22</td>
<td>11.83%</td>
</tr>
<tr>
<td>Bani malek</td>
<td>21</td>
<td>11.29%</td>
</tr>
<tr>
<td>Faifa</td>
<td>19</td>
<td>10.22%</td>
</tr>
<tr>
<td>Samttah</td>
<td>17</td>
<td>9.14%</td>
</tr>
<tr>
<td>Alahad</td>
<td>15</td>
<td>8.06%</td>
</tr>
<tr>
<td>Beash</td>
<td>14</td>
<td>7.53%</td>
</tr>
<tr>
<td>Aledabi</td>
<td>13</td>
<td>6.99%</td>
</tr>
<tr>
<td>Alreath</td>
<td>13</td>
<td>6.99%</td>
</tr>
<tr>
<td>Alaridah</td>
<td>12</td>
<td>6.45%</td>
</tr>
<tr>
<td>Abuareesh</td>
<td>11</td>
<td>5.91%</td>
</tr>
<tr>
<td>Damad</td>
<td>11</td>
<td>5.91%</td>
</tr>
<tr>
<td>Aldarb</td>
<td>9</td>
<td>4.84%</td>
</tr>
<tr>
<td>Jazan city</td>
<td>9</td>
<td>4.84%</td>
</tr>
</tbody>
</table>
Age wise distribution of cases revealed a range between <20 - >60 years with minimum number of cases 10(5.38%) in the category of >60 and maximum in the category of 20-39 years (118 cases, 63.44%) (Figure 3.1).

**Figure 3.1.** Distribution of cases as per age groups with percentage

Of the total 390 participants 316(81.03%) were males and 74(18.97%) out of 186 positive cases males constituted 148(79.57%) and females 38(20.43 %) (Fig: 3.2).

Out of 186 positive confirmed people Saudi comprised of 26(13.98%) and non-Saudi 160(86.02%), non-Saudis being 84(45.16%) Yemeni, 64(34.41%) Ethiopian, 4(2.15%) Indian, 4(2.15%) Pakistani , 2(1.08%) Sudanese and 2(1.08%) Bengali (Table 3.4 & Figure 3.3).
Figure 3.2. Distribution of gender as percentage of positive cases

Table 3.4. Nationalities of participants with percentage of positive cases

<table>
<thead>
<tr>
<th>Nationality</th>
<th>No. of positive samples</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yemen</td>
<td>84</td>
<td>45.16</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>64</td>
<td>34.41</td>
</tr>
<tr>
<td>Saudi</td>
<td>26</td>
<td>13.98</td>
</tr>
<tr>
<td>India</td>
<td>4</td>
<td>2.15</td>
</tr>
<tr>
<td>Pakistan</td>
<td>4</td>
<td>2.15</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>2</td>
<td>1.08</td>
</tr>
<tr>
<td>Sudan</td>
<td>2</td>
<td>1.08</td>
</tr>
<tr>
<td>Total</td>
<td>186</td>
<td>100.00</td>
</tr>
</tbody>
</table>
One hundred samples out of 186 (56.4%) NS1 positive cases samples tested by RT-PCR. Thirty three out of 100 (33.0%) were confirmed positive for dengue virus when using D2 and D3 primers (511bp) for all serotypes, and the RT-PCR product was used as a sample for the nested-PCR using a set of serotype-specific primers pair as described in the methodology.

Two dengue virus types (DEN-2 and DEN-3) were detected and the results showed that DEN-2 is the most common and predominant type in Jazan region rating twenty five out of thirty three (69.7%), followed by DEN-3 ten out of thirty three (30.3%), (Table 3.5).

The (DEN-2 and DEN-3) agarose gel electrophoresis with details in (Figure 3.4).
**Figure 3.4** Agarose gel electrophoresis of RT-PCR (D3, D2 primers) and nested-PCR by the specific primers. Lane 1 and 8 DNA 100bp marker, lane (2) negative control, lane (3) positive sample DEN-3, lane (4,5) positive samples DEN-2, lane (6) positive sample DEN-3 and lane (7) positive RT-PCR product sample (D3 and D2 primers for all serotypes).

![Agarose gel electrophoresis](image)

**Table 3.5. Results of RT-PCR and nested-PCR**

<table>
<thead>
<tr>
<th>RT-PCR</th>
<th>Number of positive</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>67</td>
<td>67</td>
</tr>
<tr>
<td>Type 2</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>Type 3</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

To confirm the serotype-specific results, the partial sequencing was done for thirty three RT-PCR product samples represent the two serotypes (DEN-2, and DEN-3). The Blast search showed that the sequences of our samples aligned along with many published sequences of dengue virus serotypes as shown in (Table 3.6, Figure 3.5 & Figure 3.6), and similarity tree (Figure 3.7 & Figure 3.8) which illustrates the Gen bank accession numbers and the country of isolates.
Table 3.6. Results of RT-PCR and nested-PCR

<table>
<thead>
<tr>
<th></th>
<th>DEN-2</th>
<th></th>
<th>DEN-3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Gen bank accession No</td>
<td>Country</td>
<td>Gen bank accession No</td>
<td>Country</td>
<td></td>
</tr>
<tr>
<td>JN935383</td>
<td>India</td>
<td>KM097092</td>
<td>India</td>
<td></td>
</tr>
<tr>
<td>KU351296</td>
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**Figure 3.5** Identities between DEN-2 from Jazan and DEN-2 of India(gb|JN935383.1) Dengue virus strain VCRC/DENV2/03/10 polyprotein gene, partial cds Length=508, Score = 848 bits (459), Expect = 0.0, Identities = 471/476 (99%), Gaps = 3/476 (1%), Strand=Plus/Plus

**Figure 3.6** Identities between DEN-3 from Jazan and DEN-3 of India (gb|KF954949.1) Dengue virus 3 isolates 13GDZDV30E, complete genome Length=10677 Score = 861 bits (466), Expect = 0.0, Identities = 473/476 (99%), Gaps = 1/476 (0%), Strand=Plus/Plus
Sequencing of DEN-2 in this study revealed that it is in close similarity to varies Indian types (Table 3.6 and Figure 3.5 & Figure 3.7), while DEN-3 is in similarity to some Asian types including India, China, and Singapore (Table b and Figure 3.6 & Figure 3.8).
Figure 3.7 DEN-2 serotype similarity tree.

Figure 3.8 DEN-3 serotype similarity tree.
Chapter Five

Discussion

4.1. Seasonal distribution:

The main peaks of infection during 2015 (November to December) and 2016 (January to October) included the summer. The reason for this is unclear because Jazan has a hot climate with limited rainfall. A possible explanation could be increased travel due to the annual vacation of children\(^{(92)}\). The peak of infection beginning before the summer may be due to the fact that Jeddah receives more refugees and illegal immigrants from Yemen, Ethiopia, Eritrea and Somalia from Yemen border throughout the year who are en route to inside Saudi Arabia.

4.2. Clinical features compared to US CDC definition:

The most common clinical presentations were fever, vomiting, and abdominal pain, which were also common symptoms during the first outbreak of dengue in Makah in 2004\(^{(93)}\). Fever and vomiting are symptoms associated with DF; however, protracted vomiting and severe abdominal pain may also result in the subsequent development of DSS.

In this study, we found many symptoms according to the US CDC definition of DENV infection that were either absent or present in low numbers. The relatively fewer common clinical features were hemorrhagic manifestations (associated with DHF) headaches, rash, and retro-orbital pain. Rash and hemorrhagic manifestations were also relatively uncommon in another study conducted in Jeddah\(^{(92)}\). None of the patients reported any myalgia or arthralgia in any year.

4.3 Comparison of the significant differences in clinical presentations from 2015-2016:

Significant differences were observed between the years for the percentage of patients who were IgM positive, PCR positive (indicating the presence of DENV), presenting with fever, and showed liver dysfunction.

Although there was a significant difference in nationalities from one year to another, patient nationality had no effect on the incidence of disease. No significant differences were noted...
between the years for the percentage of IgG positive patients, suggesting that there was no significant difference in the percentage of patients demonstrating a secondary antibody response. There was a slight inverse correlation of IgM positivity with patient age leading to the conclusion that the younger the patient, the greater the likelihood of a primary antibody response.

The pathogenesis of DENV is poorly understood. A complex interaction between immunopathologic, viral, and human genetic factors results in a varied DENV disease outcome\(^\text{(94)}\), which may explain the varied range of clinical presentations observed in this retrospective analysis.

A possible reason for the significant differences seen in the clinical expression of the disease between the years may be due to infection with different DENV serotypes\(^\text{(95)}\) and the possibility of concurrent infections with more than one serotype. Co-circulation of multiple DENV serotypes has been reported from many parts of the world, including India during an outbreak of DHF/DSS in 2006. Co-circulation of multiple DENV serotypes would result in an increased risk of concurrent infections. There is, however, limited documentation describing concurrent infections with more than one serotype in the same individual\(^\text{(96,97)}\). Furthermore, as already alluded to, sequential infection with more than one serotype is thought to be a major factor for the emergence of DHF\(^\text{(98)}\).

Both primary and secondary infection by any of the four DENV serotypes can cause DF and DHF; however, virus virulence is not the only factor to explain differences in host susceptibility to the disease and disease severity. Host immune response variations have been associated with polymorphism in the human genome, which may help explain why some patients develop end-stage complications in dengue disease and others only experience a mild form of the disease\(^\text{(99)}\). In another study of children with DENV infection, host genetic differences were shown to affect the immune response and consequently, influence disease outcome\(^\text{(100)}\).

Jazan region has witnessed several outbreaks during the recent decade (290 cases in 2010, 289 cases in 2012, and 555 cases in 2016 – Dengue control program in Jazan). The current available data on dengue in Jazan has concentrated mainly on serological surveys (Al-Arzaqi et al., 2013; Gamli et al., 2014)\(^\text{(101,102)}\) and has not analyzed the circulating serotypes in the region.
Our results indicated that dengue fever is becoming highly prevalent in Jazan region (47.69 %) compared to the previous reports of (Al-Arzaqi et al., 2013; Gamil et al., 2014)\(^{(101,102)}\) who reported dengue prevalence of 26.5% and 47.74% , respectively, in the region. In this study, two dengue virus types (DEN-2 and DEN-3) were found circulating in Jazan region with the predominance of DEN-2 scoring 23 out of 33 dengue positive RT-PCR samples (69.7%), followed by DEN-3 (10 out of 33 – 30.3%), how-ever serotype 1 and 4 was not detected in any of the 100 dengue cases.

This finding is in complete accordance with the work of Fakeeh and Zaki (2001)\(^{(83)}\) who reported that DEN-2 was the predominant serotype, followed by DEN-1, and DEN-3 in Jeddah, Saudi Arabia. Whereas Organji et al (2017)\(^{(103)}\) in Makah city, showed that DEN-1 was the predominant dengue virus type, followed by DEN-2 and then DEN-3, although the positive blood samples they used were only six.

The results also coincide partially with the findings of Khan et al (2008)\(^{(104)}\) who reported high prevalence of the DEN-2 in contrast to the prevalence of DEN-1 found by Organji et al (2017)\(^{(103)}\) in Makah city. In Jeddah, Zaki et al (2008)\(^{(85)}\) revealed that DEN-1 and DEN-2 caused the major outbreak in 1994, while DEN-3 emerged in 1997. More-\%ever, they indicated two genotypes for DEN-1 (America-Africa genotype, and Asia-2 genotype), DEN-2 genotype clustered within Cosmopolitan genotype, and DEN-3 clustered within genotype III.

In the present study, we found DEN-2 to be the predominant dengue virus type, a result which is in line with the reports of Fakeeh and Zaki (2001, 2003) and Zaki et al. (2008)\(^{(83,85)}\) who stated that DENV-2 virus is the predominant serotype in Saudi Arabia particularly in western Saudi Arabia since 1992. El-Kafrawy et al. (2016)\(^{(105)}\) showed that DEN-2 isolate from Jeddah belongs to the Cosmopolitan genotype was most genetically related to isolates from Pakistan circulating from 2008 to 2013. The three dengue virus serotypes DEN-1, DEN-2, and DEN-3 are thought to be predominant in the Middle East, especially in Yemen and Saudi Arabia\(^{(106)}\).

Dengue viruses circulating locally in Saudi Arabia are likely to have been imported into Saudi Arabia by Saudi traveling abroad to dengue endemic countries, or during Hajj and Umrah seasons, or by migrant labour\(^{(85)}\). The introduction of the two dengue virus types in Jazan region may be resulted from several factors; traveling of the Jazan citizens for Hajj and Umrah or for
trade or other purposes, or by traveling abroad to dengue endemic countries, or by migrant labour, or due to the proximity of Jazan region to Yemen where the disease is endemic and the two dengue virus serotypes DEN-2, and DEN-3 are circulating.

The similarity of DEN-2 to varies Indian types, in addition to, the similarity of DEN-3 to some Asian types including India, China, and Singapore suggested the likelihood of introduction of these serotypes to Jazan region either by travel-ling from and to those countries especially the migrant labours (DEN-1,DEN-2, DEN3,), or through direct introduction from Jeddah (DEN-1 Jeddah genotype) and Yemen which is closet to Djibouti and Eritrea (DEN-1 African origin).

It is stated that shifts in circulating dengue virus type or introduction of new dengue virus type in endemic areas have shown to be related with incidence of severe dengue infections; DHF and DSS\(^{40,73}\). Moreover, it is worthy to note that the primary infections by DEN-1 and DEN-3 are related with more dengue sever infections, whereas infections with DEN-2 and DEN-4 are associated with increased dengue severity when they present as secondary infections\(^{107}\). Results of our study have analyzed multiple dengue serotypes which would help in providing clear evidence of current active dengue transmission and endemicity in Jazan region.
Chapter Six

Conclusion and Recommendations

Conclusion:

Unfortunately, the dengue surveillance systems in middle east and probably in other developing countries in the world are not structured to either prevent dengue epidemic or improve endemic situations. Basically their function and goals are to detect epidemics in the initial stage to prepare hospitals, health workers and communities in response to that situation. However, real advantages of new surveillance strategies are not being well spent to improve the control and prevention of dengue disease.

The results of this study reported for the first time the dengue virus types DEN-2, and DEN-3 circulating in Jazan region with the DEN-2 being the pre-dominant one. The high seroprevalence of dengue virus infection in Jazan region indicates its endemicity. The present study highlights the importance of tracking the spread of dengue virus types and its implication for analyzing changes in dengue endemicity in specified areas over time. Continuous surveillance of dengue virus serotypes in the region to detect as earlier the local origin circulating serotypes from the imported ones especially new types DEN-4 and DEN-5, for which continued surveillance is imperative. Complete genome sequencing is required for the two detected dengue virus serotypes circulating in the region (DEN-2, and DEN-3) to serve as references for any future epidemiological researches and outbreaks.

Dengue infection can have potentially fatal consequences, and to date, vector control methods to prevent the spread of the virus have been unsuccessful (108). Although there are promising vaccine candidates in development, further studies are required for a greater understanding of the humoral immune responses to DENV infection and disease pathogenesis (109).
Recommendations

- In the surveillance system health workers should be ready to differentiate the symptoms when the patients are coming in early or in late acute phase of the dengue disease.
- In a near future we have to include new procedures in the surveillance of dengue and this study was oriented to help in that sense.
- Future studies have to include children less than 5 years old, by no published reports we know that a high proportion of children was born with mother IgG dengue antibody and it should be affecting the crude incidence of the disease.
- In our opinion, public health surveillance, in a big cities, should have at least two samples of population (with high and low incidence), like sentinel surveillance. It could detect new changes in the virus transmission, changes in the expected incidence and validate information obtained in massive and passive surveillance system.
References


