PREVALENCE OF DENGUE VIRUS IN MOSQUITOES CAUGHT IN OYE EKITI, EKITI STATE, NIGERIA.

BY:

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MCB/11/0343

A PROJECT WORK

IN PARTIAL FULFILMENT OF THE AWARD OF BACHELOR OF SCIENCE (B.Sc) DEGREE IN MICROBIOLOGY

SUBMITTED TO

THE DEPARTMENT OF MICROBIOLOGY

FEDERAL UNIVERSITY, OYE EKITI

EKITI STATE,NIGERIA

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OCTOBER, 2015.

CERTIFICATION PAGE

This is to certify that this project work was carried out by Olorunfemi Olatunbosun Adedayo from Department of Microbiology, Faculty of Science, Federal University Oye Ekiti under my supervision and it is a fair reflected to the student input.

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DEDICATION

This work is dedication to the Almighty God for his ever sufficient Grace and Mercy over me.

ACKNOWLEDEGEMENT

I sincerely express my gratitude to God Almighty, who gave me, life, good health, sound mind, finance and literary ability to carry out this research work.

I wish to convey my deep appreciation to my supervisor Dr. Lawrence Okoror, who guided me and despite his tight schedule made out time to read and correct my manuscript, his contribution will always be an imprint in my mind.

I also wish to express deep appreciation to my father Hon.Tunde Olorunfemi who has always being the source of encouragement to me and to my mother Mrs. Florence Olorunfemi who gave me support towards my being what I am today, I say big Thank you.

I would say big thank you to Engr.Richard Adu, Mrs.Christana Adu, Mrs.Omolara Falokun, and Mrs.Aina Fasanmi for their open support throughout my stay in Federal University Oye Ekiti…God bless you.

My sincere appreciation to my sister, Olorunfemi Moyinoluwa and my brothers Ayodeji and Omotolani Olorunfemi for their love, care and support thoughtout my programme in Federal University Oye Ekiti, you are all wonderful.

To all my lecturers, the admin staffs and technologist in the department of Microbiology, Federal University Oye Ekiti, I say big thank you may the good Lord bless you all.

I bow with my cap raised high to salute the scholars and authors whose ideas have been gathered together in this research work. Thank you.

Olorunfemi Olatunbosun Adedayo.

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ABSTRACT

Dengue Virus is the most prevalent arthropods borne viral infection in tropical and sub-tropical regions worldwide and they are transmitted by *Aedes aegypti* and *Aedes albopictus*. This experiment was conducted with the aim of detecting and establishing possible sylvatic transmission of Dengue Virus in mosquitoes caught in Oye Ekiti. Mosquito life traps were used to trap 200 Aedes mosquitoes which were then knocked down by the use of insecticides. RNA were extracted from the mosquitoes and amplified using PCR machine. The amplified RNA were loaded into gel and electrophoresis machine was used to test for the presence of Dengue Virus in the sample. In all the mosquitoes tested, no Dengue Virus was detected.

CHAPTER ONE

1.0 INTRODUCTION

Dengue virus is a positive single-stranded RNA virus with an icosahedral nucleocapsid surrounded by an envelope. Dengue virus of the genus Flavivirus which belong to the family Flaviviridae (Rodenhuis *et al.,* 2010). Dengue virus is composed of four serotypes which are; DENV-1. DENV-2, DENV-3 and DENV-4. Dengue virus is an arbovirus and it is transmitted by Aedes mosquito which are; *Aedes aegypti* and *A. albopictus*.

Aedes mosquito which belongs to the family of Culicidae serves as primary vector for dengue virus. They are small, dark mosquito with conspicuous white markings and banded legs, a black proboscis and white scaling on the tips of the palps (Russell *et al.,*2005).They prefer to bite indoors and primarily bite humans. Aedes mosquitos bite during the day and are especially active in the morning between 6-7am and in the late afternoon 5-6pm. They bite humans, however it will feed on the wide range of species including birds and mammals.

Dengue virus is the one of the most important arboviruses causing human disease(Gubler, 2002).The first reported epidemic of dengue fever (DF) occurred in 1779–1780 in Asia, Africa, and North America(Burke and Monath,2001).Dengue virus causes dengue fever(DF), dengue hemorrhagic fever (DHF) and Dengue shock

syndrome(DSS) in human when infected Aedes mosquito bite uninfected person. The symptoms of dengue fever includes; high body temperature of about 41^0C, headache, pain behind the eyes, bone, muscle and joint pain.

Dengue infections can be prevented by wearing protective clothing and using a mosquito repellant throughout the day. It can be control by the complete eradication of Aedes mosquito and larva in the environment by controlling vector populations by various methods which includes; physical means which is done by the removal of breeding places, chemical means which deals with the use of chemicals such as insecticides and larvicides and the biological means can be done by using bacteria such as *Bacillus thuringiensis* to control the transmission of the disease. There is no specific medication yet for dengue infections.

Dengue epidemics require a coincidence of large numbers of Aedes mosquito and large numbers of people with no immunity to one of the four distinct types (DENV-1 to DENV-4). Dengue occurs in an area that has the combination of warm and humid climate and overcrowding and major urban centers.

CHAPTER TWO

LITERATURE REVIEW

2.0 DENGUE VIRUS

Dengue virus is a mosquito-borne positive single-stranded-RNA virus of the family *Flaviviridae*; genus *Flavivirus* (Rodenhuis *et al.*,2010). This genus also includes West Nile virus, tick borne virus, encephalitis virus, yellow fever virus and other virus which may cause encephalitis. The virus is made of four serotypes which are; DENV-1. DENV-2, DENV-3 and DENV-4.All four serotypes can cause the full spectrum of disease (Rodenhuis *et al.*,2010).

2.1 STRUCTURE OF DENGUE VIRUS

Dengue virus is a positive single-stranded RNA virus. Its genome is about 11000 bases in length that codes for three structural proteins, capsid protein C, membrane protein M, envelope protein E; with seven non-structural proteins which are; NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5; and short non-coding regions on both the 5' and 3' ends (Rodenhuis *et al.*,2010). The virus in an electron micrograph consists of 40-50nm sphere, surrounded by a lipopolysaccharide envelope.

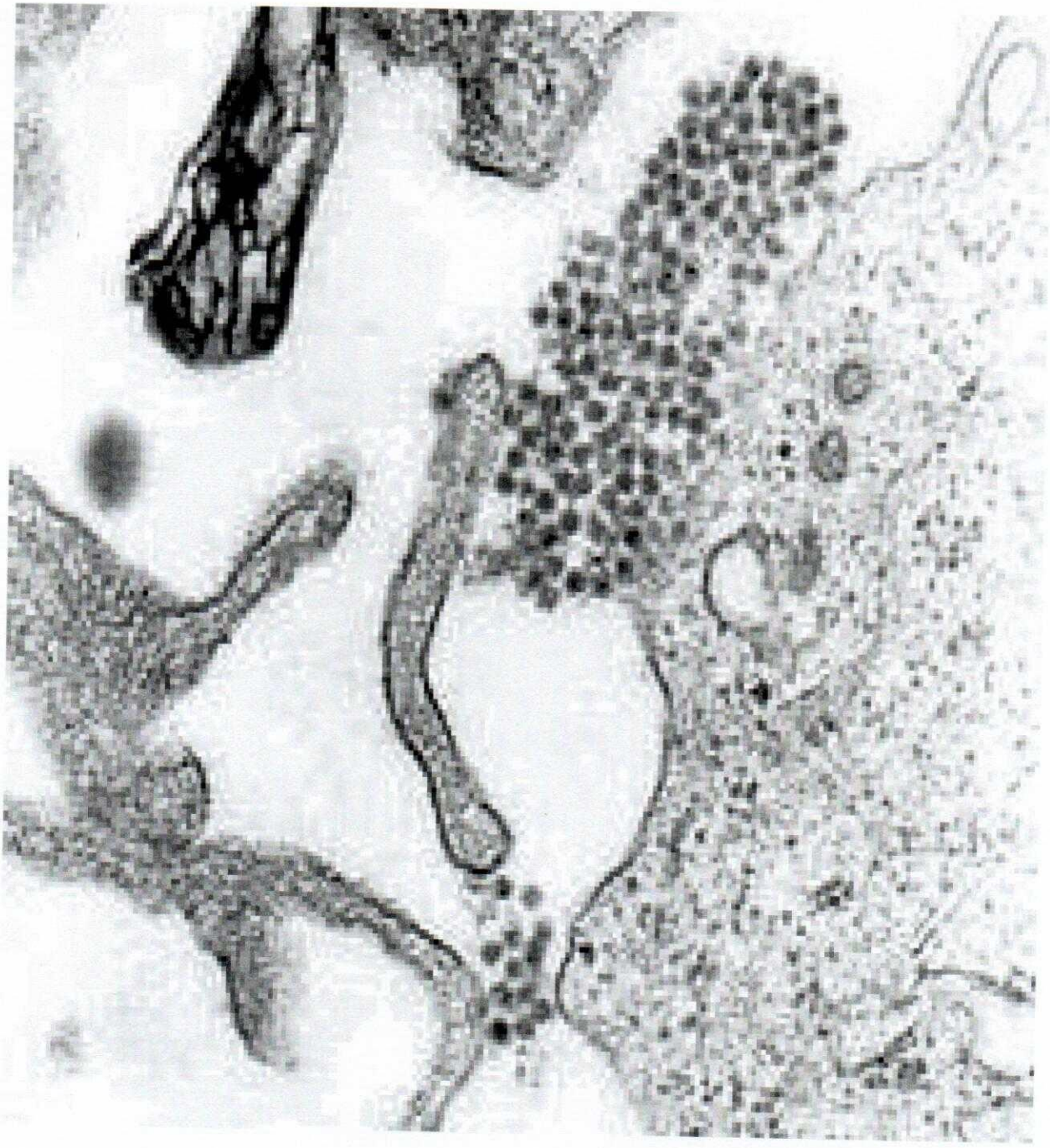

Figure1: A TEM micrograph showing dengue virus virions. Source: Wikipedia

2.2 EVOLUTION OF DENGUE VIRUS

Several epidemic occurred between 1635–1699 in the Caribbean, Asia, Africa, and North America. During this period, sailing vessels carrying mosquitos were primary means of transporting this vector from endemic regions to new areas.

During the Philadelphia epidemic of 1779–1780, Dr.Benjamin Rush first described the dramatic symptoms of dengue as "Break bone Fever." Jump forward to the late 1930s, where World War II facilitated spread of dengue through Asia/Pacific region. Approximately 80,000 military personnel serving in the Pacific theater were diagnosed with dengue between 1942 and 1945.

The dengue type 1 virus appears to have evolved in the early 19th century (Patil *et al.*,2011). Based on the analysis of the envelope protein there are at least four genotypes (1 to 4). The rate of nucleotide substitution for this virus has been estimated to be 6.5×10^{-4} per nucleotide per year, a rate similar to other RNA viruses. The American African genotype has been estimated to have evolved between 1907 to1949. This period includes World War I and II which were associated with considerable movement of populations and environmental disturbance, factors known to promote the evolution of new vector borne viral species.

2.3 LIFE CYCLE OF DENGUE VIRUS

Dengue virus was transmitted in sylvatic cycles in Africa and Asia between mosquitoes of the genus *Aedes* and non-human primates with rare emergences into human populations (Holmes *et al.*,2013). The global spread of dengue virus, however, has followed its emergence from sylvatic cycles and the primary life cycle now exclusively involves transmission between humans and *Aedes* mosquitoes (Halstead, 2013).Vertical transmission from mosquito to mosquito has also been observed in some vector species (Haddow *et al.*,2013).

Susceptible humans become infected after being bitten by an infected female Aedes mosquito. Viremia in humans begins toward the end of a four- to six-day incubation period and persists until fever abates, which is typically three to seven days (Stramer *et al.*,2012). An uninfected Aedes mosquito may acquire the virus after feeding during this viremic period. The mosquito has an incubation period of 8 to 12 days before it is capable of transmitting the virus to susceptible individuals. Once infected, mosquitoes carry the virus for their lifespan and remain infective for humans.

2.4 DENGUE FEVER

Dengue fever is an emerging infectious disease increasing in prevalence in many geographic regions, including the Caribbean. It is the most common arboviral (vector-borne) disease in the world, and infects more that 50 million people annually worldwide. The etiological agent of dengue fever is one of four serotypes of the dengue virus (DENV1–DENV4). The infection is transmitted via a human-mosquito-human route, when one or more species of the *Aedes* mosquito takes a blood meal from an infected host and then feeds on a person who is uninfected. There is no vaccine or cure for dengue fever. The exact pathophysiology of severe dengue infection (dengue hemorrhagic fever and dengue shock syndrome) is still an enigma, although it is now widely accepted that the host immune system, host genetic makeup, and pathogen virulence all contribute towards the rapid deterioration seen in some patients(Sanchez *et al.*,2006)

2.5 HISTORY OF DENGUE FEVER

The first record of a case of probable dengue fever is found in a Chinese medical encyclopedia from the Jin Dynasty (265-420 AD) which referred to as water poison associated with flying insects (Gubler, 1998). The primary vector is *Aedes aegypti* and *A. albopictus* spread out of Africa in the 15th to 19th centuries due to increased globalization secondary to the slave trade. There has been description of epidermics

in the 17th century, swept Asia, Africa and North America. From that time until 1940. Epidermics were in frequent (Gubler, 1998).

In 1906, transmission by the Aedes mosquitoes were confirmed, and in 1907 dengue was the second disease after yellow fever that was shown to be caused by a virus. Further investigation by John Burton Cleland and Joseph Franklin Siler Completed the basic understanding of dengue transmission. The marked spread of dengue during and after the Second World War has been attributed to ecological disruption. The same trends also led to the spread of different serotypes of the disease to new areas, and to the emergence of dengue hemorrhagic fever (Gubler, 1998).

This severe form of the disease was first reported in the Philippines in 1953; by the 1970s, it had become a major cause of child mortality and had emerged in the Pacific and Americans. Dengue hemorrhagic fever and dengue shock syndrome were first noted in Central and South American in 1981, as DENV-2 was contacted by people who had previously been infected with DENV-1 several years earlier (Gould *et al.*, 2008) .

2.6 CLASSIFICATION OF DENGUE FEVER

The world health organization's 2009 classification divides dengue fever into two groups: uncomplicated and severe (WHO, 2009). This replaces the 1997 WHO classification, which is needed to be simplified as it had been found to be restrictive, though the older classification is still widely used.

Severe dengue is associated with severe bleeding. Severe organ dysfunction, or severe plasma leakage while all other cases are uncomplicated. The 1997 classification divided dengue into undifferentiated fever, and dengue hemorrhagic fever. Dengue hemorrhagic fever was subdivided further into grades I-IV.

Grade I is associated with the presences of easy bruising or a positive tourniquet test in someone with fever. Grade II is associated with the presence of spontaneous bleeding into the skin and elsewhere. Grade III is the clinical evidence of shock, and Grade IV is shock so severe that blood pressure and pulse cannot be detected. Grade III and IV are referred to as ''dengue shock syndrome'' (WHO, 1997).

2.7 ETYMOLOGY OF DENGUE FEVER

The origin of the word "dengue'' are not clear, but one theory is that it is derived from the Swahili phrase Ka-dinga pepo, which describe the disease as being caused by an evil spirit. The Swahili word dinga may possibly have its origin in the suffering the bone pain of dengue fever. (Harper, 2001). However, it is possible that the use

of Spanish word derived from the similar-sounding Swahili. Slave in the West Indies having contracted dengue were said to have the posture and gait of a dandy, and the disease was known as "dandy fever". The term "break bone fever" was first applied by physician and united state founding father Benjamin Rush, in a 1789 report of the 1780 epidemic in Philadelphia. In the report he use in primarily the more formal term "bilious remitting fever". The term dengue fever came into general use after 1828. Other historical term include "break heart fever" and "la dengue". Term for severe disease include "infectious thrombocytopenic purpura" and 'Philippine', "Thai" or Singapore hemorrhagic fever (Halstead, 2008).

2.8 EPIDEMIOLOGYOF DENGUE FEVER

Most people with dengue fever recover without any ongoing problem (WHO, 2009). The mortality is 1-5% without treatment, less than 1% with adequate treatment; however severe disease carries mortality of 26%. Dengue is endemic in more than 110countries. It infects 50-100million people worldwide in a year, leading to half a million hospitalizations, and approximately 12,500-25,000 death (Varatharaj, 2010. The most common disease transmitted by arthropods, dengue has disease burden estimated to be 1600 disability-adjusted life years per million population. Which is similar to tuberculosis, another childhood and tropical diseases. As a tropical disease dengue fever is deemed only second in importance to malaria, through the World Health Organization counts dengue as one of sixteen neglected tropical disease

(WHO, 2009). The incidence of dengue increased 30 fold between 1960-2010. This increase is believed to be due to a combination of urbanization, population growth, increased international travel and global warming. The geographical distribution is around the equator with 70% of the total 2.5billion people living in endemic area from Asia and the pacific (WHO, 2009). In the United States, the rate of dengue infection among those who return from an endemic area with fever is 2.9-8.0 and it is the second most common infection after malaria to be is diagnosed in this group (Chen *et al.*,2010).

Like most arboviruses, dengue virus is maintained in nature in cycles that involves preferred blood-sucking vectors and vertebrate hosts. The viruses are maintained in the forests of South-East Asia and Africa by transmission from female Aedes mosquitoes of species other than *A.aegypti* to her offspring and to lower primates. In towns and cities, the virus is primarily transmitted by, the highly domesticated *A.aegypti.* In rural settings the virus is transmitted to humans by *A.aegypti* and other species of Aedes such as *A.albopictus*. Both these species have expanding ranges in the second half of the 20th century. In all settings the infected lower primates 'human greatly increase the number of circulating dengue viruses in a process called amplification (Gubler, 2010). Infections are most commonly acquired in the urban environment. In recent decades, the expansion of villages, town and cities in endemic areas, and the increased mobility of people have increased the number of

epidemics and circulating virus. Dengue fever, which was once confined to South-East Asia now spread to South-East China, countries in the Pacific Ocean and America and might pose a threat to Europe.

Dengue virus has emerged in recent decades as a worldwide public health problem, particularly in the Asia-Pacific and public health effect of dengue is not clear dangerously low blood pressure occurs. Aedes species mosquitoes are widely distributed in Africa and can serve as vector of dengue virus (DENV). When their distribution is combined with rapid population growth, unplanned urbanization and increased international travel, extensive transmission of DENV is likely in Africa. Over the past 5 decades, cases of epidemic or sporadic dengue hemorrhagic fever (DHF) have been reported in few countries in Africa, no outbreak have been reported. However, when compared with the Asia-Pacific and Americans-Caribbean region, the epidemiology and effects not been defined. A dengue outbreak in Cape Verde was recently reported (>3,000 case), and the reappearance of dengue in Senegal after 20years was also reported (Gubler, 2010).

2.9 TRANSMISSION OF DENGUE FEVER

Dengue virus is primarily transmitted by Aedes mosquitoes particularly *A.aegypti.* These mosquitoes usually live between the latitudes of 35 North and 35 South below an elevated of 1,000 meters (3,000ft) (WHO, 2009). They are active in the early

morning and in the evening. Other Aedes species that transmit the disease include *A.albopictus, A.polynesienes and A.scutellaris*. Human are the primary host of the virus, but it also circulates in non-human primates. An infection can be acquired via a single bite. A female mosquito that takes a blood meal from a person infected with dengue fever becomes itself infected with the virus in the lining its gut. About 8-10days later, the virus spread to other tissues including the mosquito's salivary glands and is subsequently released into saliva. The virus seems to have no dentrimental effect on the mosquito, which remains infected for life. *Aedes aegypti* prefers to lays its eggs in artificial water containers, to live in close proximity to human and to feed on people rather than other vertebrates (Wiwanitkit, 2010).

Dengue can also be transmitted via infected blood products and through organ transplanting. In countries such as Singapore, where dengue is endemic, the risk is estimated to be between 1.6 and 6per 10,000 transfusions. Vertical transmission (from mother to child) during pregnancy or at birth has been reported (Wiwanitkit, 2010). Other mean of transmission is person to person mode of transmission have also be reported, but are very unusual. The genetic variation in dengue viruses region specific; suggestive that establishment into new territories is relatively infrequent, despite dengue emerging in new region in recent decades. Dengue is transmitted by several species of mosquito within the genus Aedes, principally *Aedes aegypti.* The virus has four different types; infection with one type usually gives long life

immunity to that type increased the risk of severe complications. As there is no commercially available vaccine, prevention is sought by reducing the habitat and the number of mosquitoes and limiting exposure.

2.10 CLINICAL MANIFESTATION OF DENGUE FEVER

The characteristics symptoms of dengue fever are sudden-onset fever, headache (typical location behind the eye), muscle and joint pains, and rash. The alternative name for dengue, 'break bone fever', comes from the associated muscle and joint pains. The course of infection is divided into three phases: febrile, critical, recovery (WHO, 2009).

2.10.1 FEBRILE PHASE

The febrile phase involves high fever, often over 40^0C (104F) and is associated with generalized pain and headache; this is usually lasts two to seven days (2-7days). Vomiting may also occur. A rash occurs in 50-80% of those symptoms in the first or second day of symptoms as flushed skin, or later in the course of illness (day4-7), as measles-like rash. Some patchier (small red spots that do not disappear when the skin is pressed, which are caused by broken capillaries) can appear at this point, as may some mild bleeding from the mucous membranes of the mouth and nose. The fever itself is classically biphasic in nature, breaking and then returning for one or

two days, although there is wide variation in often this pattern actually happens, (Knoop *et al.*,2010).

2.10.2 CRITICAL PHASE

In some people, the disease proceeds to a critical phase around the time fever resolves and typically lasts one or two days. During this phase there may be significantly fluid accumulation in the chest and abdominal cavity due to increased capillary permeability and leakage. This leads to depletion of fluids from the circulation and decreased blood supply to vital organs. During this phase, organ dysfunction and severe bleeding, typically from the gastrointestinal tract, may occur. Shock (dengue and syndrome) and hemorrhage (dengue hemorrhagic fever) occur is less than 5% of all cases of dengue, however those who have previously been infected with other serotypes of dengue virus (secondary infection) are at an increased risk. This critical phase, while rare occur relatively more commonly in children and young adults (Varatharay, 2010).

2.10.3 RECOVERY PHASE

The recovery phase occurs next, with resumption of the leaked fluid into the bloodstream. The improvement is often striking, but there may be severe itching and a slow heart rate. Another rash may occur with either a macolopapular or vacuities appearance, which is followed by peeling of the skin. During this stage, a fluid

overload state feeling may occur; if it affects the brain, it may cause a reduced level of consciousness or seizures. A feeling of fatigue may last for weeks in adults (Ranjit *et al.*,2010).

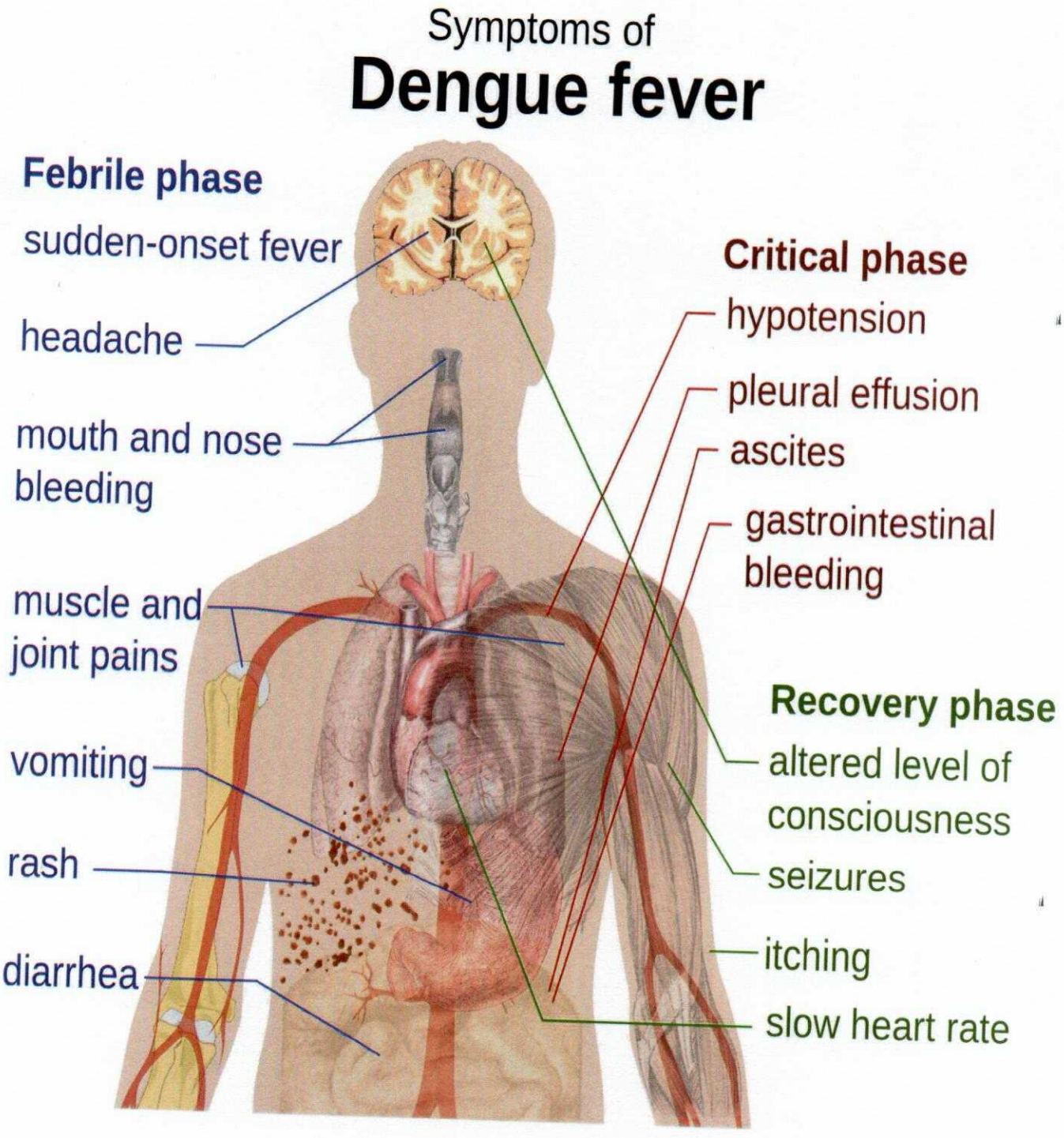

Figure 2: Schematic depiction of the symptoms of dengue virus

Source: Wikipedia

2.11 MECHANISMS OF INFECTION

When a mosquito carrying dengue virus bite a person, the virus enters the skin together with the mosquito's saliva. It binds to and enters the white blood cells, and reproduces inside the cells while they move throughout the body. The white blood correspond by producing a number a signaling proteins, such as cytokines and interferon's which are responsible for many of the symptoms, such as fever, the flu-like symptoms and the severe pains. In severe infection, the virus producing inside the body is greatly increased and many more organs (such as liver and the bone marrow) can be affected and fluids from the bloodstream leaks through the wall of small blood vessels into body cavities. As a result of this, less blood circulates in blood vessels and the blood pressure becomes so low that it cannot sufficient blood to vital organs.

Furthermore, dysfunction of the bone marrow leads to reduced numbers of platelets which are necessary for effective blood clotting; this increases the risk of bleeding, the other major complication of dengue fever.(Martina *et al.*,2009).

2.11.1 VIRAL REPLICATION

Once inside the skin, dengue virus binds to Langerhans cells (a population of dendritic cells in the skin that identifies pathogens). The virus enters the cell through binding between viral proteins and membrane proteins on Langerhans cells,

specifically the C-type lectins called DC-SIGN, mannose receptor and CLEC5A. DC-SIGN, a non-specific receptor for foreign material on dendrite cell, seems to be the main point of entry. The dendrite cell moves to the nearest lymph node. Meanwhile, the virus genome is translated in membrane-bound vesicles on the cell's endoplasmic reticulum, where the cell's protein synthesis apparatus produces new viral proteins that replicate the viral RNA and begins to form viral particles. Immature virus particles are transported to the Golgi apparatus, the part of the cell where some of the proteins receive necessary sugar chains (glycoproteins). They now mature to new virus bud on the surface of the infected cell and are released by exocytose. They are able to enter other blood cells, such as monocytes and macrophages (Rodenhuis *et al.*, 2010).

The initial reaction of infected cells is to produce interferon, a cytokines that raises a number of defenses against viral infection through the innate immune system by augmenting the production of a large group of proteins mediated by the JAK-STAT pathway. Some serotypes of dengue virus appear to have mechanism to slow down this process. Interferon also activates the adaptive immune system which leads to the generation of antibodies against the virus as well as T-cells that directly attach any cell infected with the virus. Various antibodies are generated; some bind closely to the viral proteins and target them for phagocytosis (ingestion by specialized cells and destruction), but some bind the virus less well and appear instead to deliver the

virus into a part of the phagocytes where it is not destroyed but is able to replicate further (Rodenhuis *et al.*,2010)

2.11.2 SEVERE DISEASE

It is not entirely clear why secondary infection with a different strains of dengue virus infection places people at risk of dengue hemorrhagic fever and dengue shock syndrome. The most widely accepted hypothesis is that of antibody-dependent enhancement (ADE). The exact mechanism behind the ADE is unclear. It may be caused by poor binding of non-neutralizing antibodies delivery into the wrong compartment of white blood cells that have ingested the virus for destruction. There is a suspicious that ADE is not the only mechanism underlying severe dengue-related complications and various lines of research have implied a role for T –cells soluble factors such as cytokines and the complement system. (Martina *et al.*,2009)

Severe disease is marked by the problems of capillary permeability (an allowance of fluid and protein normally contained within the blood to pass) and disordered state of the endothelial glycocalyx which acts as a molecular filter of blood components. Leaky capillary (at the critical phase) are thought to be caused by decreased in the viral load of blood that triggers an immune system response. Other processes of interest include infected cells that become necrotic which affect both coagulation

and fibrinolysis (opposing systems of blood clotting and clot degradation) and low platelets in the blood, also a factor is normal clotting (Chen *et al.*,2010).

2.11.3 ASSOCIATED PROBLEMS

Dengue can occasionally affect several other body system, either in isolation or along with the classic dengue symptoms. A decreased level of consciousness occurs in 0.5-6% of severe cases, which is attributed either to infection of the brain by the virus or directly as a result of impairment of vital organs, for example the liver (Varatharay, 2010)

Other neurology disorder has been reported in the context of dengue, such as transverse myelitis and Guillain-Barre syndrome. Infection of the heart and acute liver failure are among the heart and acute liver failure are among the rarer complications (Ranjit *et al.*,2010)

2.11.4 PREDISPOSITION

Polymorphisms (normal variation) in particular gene have been linked with an increased severe dengue complications. Examples include the gene coding for the proteins known as TNF, manna-binding lectin,CTLA4, TGF,DC-SIGN,PLCE1 and particular form of human leukocyte antigen from gene variation of HLA-B a common genetic abnormality in Africans known as glucose-6-phosphate dehydrogenase deficiency, appears to increase the risk. Polymorphism in the gene

for the vitamin D receptor and FeyR seem to offer protection against severe disease in secondary dengue infection (Whitehorn *et al.*,2010).

2.12 DIAGNOSING DENGUE FEVER

The diagnosis of dengue is typically made clinically, on basis of reported syndrome and physical examination; this applies especially in endemic areas. However, early disease can be difficult to differentiate from other viral infections. A probable diagnosis is based on finding of fever plus two of the following nausea and vomiting, rash, generalized pains, low white blood cell count, positive tourniquet test, or any warning sign in someone who live in an endemic area. Warning sign typically occur before the onset of severe dengue. The tourniquet test, which is particularly useful in setting where no laboratory investigations are readily available, involves the application of blood pressure cuff at between the diastolic and systolic pressure for five minutes, followed by the counting of any petechial hemorrhages; a higher number makes a diagnosis more likely (Halstead, 2008).

The diagnosis should be considered in anyone who develops a fever within two weeks of being in the tropics or subtropics. It can be difficult to distinguish dengue and chikungunya, a similar viral infection that shares many symptoms and occurs similar parts of the world to often investigations are performed to exclude other

conditions that causes similar symptoms, such as malaria, leptospirosis fever, typhoid fever, meningococcal disease, measles and influenza (WHO, 2009).

The earliest change detectable on laboratory investigations is a low white blood cell counts, which may then be followed by low platelets and metabolic acidosis. A moderately elevated level of amino transferees from the liver is commonly associated with low platelets and white blood cells. In severe disease, plasma leakages result in hem concentration (as indicated by a raising hematocrit) and hypoalbuminemia. Pleural effusions or ascites can be detected by physical examination when large, but the demonstration of fluid on ultrasound may assist in early identification of dengue shock syndrome (Whitehorn *et al.*,2010). The use of ultrasound is limited by lack of availability in many settings. Dengue shock syndrome (DSS) occurs if pulse pressure drops to 20mmHg while peripheral vascular collapse is evidenced (Simmons *et al.*,2012).

2.12.1 LABORATORY TEST.

When laboratory test for dengue fever become positive where zero is the start of symptoms.

First refers are those with primary infection and second refers are those with secondary infection.

Dengue fever may be diagnosed by microbiological laboratory testing. This can be done by virus isolation in cell cultures, nuclei acid detection by PCR, viral antigen detection (such as NS1) or specific antibodies (serology) (Guzman *et al.*,2010). Virus isolation and nucleic acid detection are more accurate than antigen detection but these test are not widely available due to their greater cost. Detection of NS1 during the febrile phase of a primary infection may be greater than 90% however is only 60% in subsequent infection.

All tests may be negative in early stages of a disease. PCR and viral antigen detection are more accurate in the first seven days. In 2012, a PCR test was introduced that can run on equipment used to diagnose influenza; this is likely to improve access to PCR based diagnosis according to new CDC test that was carried out in 2012.

These laboratory tests are only of a diagnostic value during the acute phase of illness with the exception of serology test for dengue virus specific antibodies, types IgG and IgM can be useful in confirming a diagnosis in the later stages of the infection. Both IgG and IgM are produced after 5-7days. The highest levels of IgM are detected following a primary infection, but IgM is also produced in secondary and tertiary infections. IgM becomes undetectable in 30-90days after a primary infection, but earlier followings. Re-infections IgG by contrast, remains detectable for over 60years and in the absence of symptoms, is a useful indicator of past infection.

After a primary infection IgG reaches peak levels in the blood after 14-21days. In subsequent re-infection, levels peak earlier and the titers are usually higher. Both IgG and IgM provide protective immunity to the infecting serotype of the virus. The laboratory test for IgG and IgM antibodies can cross-react with other flavivirus and may result in a false positive result after recent infection or vaccination with yellow fever virus or Japanese encephalitis (Whitehorn et al.,2010). The detection of IgG alone is not considered diagnostic unless blood samples are collected 14days apart and a greater than fourfold increase in levels of specific IgG is detected. In a person with symptoms, the detection of IgM is considered diagnostic (Gubler, 2010).

2.13 PREVENTION AND CONTROL OF DENGUE FEVER

Dengue fever can be prevented and controlled by two means which are;

1. Mosquito control: Mosquito can be controlled by the use of chemicals such as insectides on adult mosquito and larvicides on mosquito larva and also clearing of bushes and avoiding stagnant water around residential area. All breeding places must be removed by throwing away or recycle water-holding containers that are not needed and also if empty containers must be stored, they should be covered, turned over or placed under a roof that does not allow them to fill with water.

2. Avoid mosquito bites: This can be done by wearing long sleeve shirts, long pants, socks and shoes when mosquitoes are most active. Apply repellents such as DEET, picaridin, oil of lemon eucalyptus. Do not use repellents under clothing. Also, Use mosquito netting over infant carriers, cribs and strollers and install or repair window and door screens to keep out mosquitoes.

2.14 TREATMENT AND MANAGEMENT

There are no specific antiviral drugs for dengue fever, however maintaining proper fluid balance is important. Treatment depends on the symptoms, varying from oral hydration therapy at home with close follow-up, to hospital admission with administration of intravenous fluids and or blood transfusion. Intravenous hydration is usually only needed for one or two days. The rate of fluids administration is titrated to a urinary output of 0.5-1mL/kg/hr., stable vital sign and normalization of hematocrit (Ranjit *et al.*, 2010).

Invasive medical procedures such nasogastric intubation, intramuscular injections and arterial punctures are avoided in view of the bleeding risk. Paracetamol (acetaminophen) is used for fever and discomfort while NSAIDs such as ibuprofen and aspiring are avoided as they might aggravate the risk of bleeding. Blood transfusion is initiated early in patients presenting with unstable vital signs in face of a decrease to some predetermined "transfusion trigger" level. Packed red blood

cell or whole bloods are recommended, while platelets and fresh plasma are usually not.

During the recovery phase intravenous fluid discontinued to prevent a state of fluid overload. If fluid overload occurs and vital sign are stable, stopping further fluid may be all that is needed. If a person is outside of the critical phase, a loop diuretic such as furosemide may be used to eliminate excess fluid from the research effort to prevent and treat dengue including various means of vector control, vaccine development and antiviral drugs (Varatharay, 2010).

With regard to vector control, a number of novel methods have been used to reduce mosquito number with some success including the placement of the guppy (Poecilia reticulate) or copepod in stagnant water to eat the mosquito larvae. Attempts are ongoing to infect the mosquito population with bacteria of the Wolbachia genus, which makes the mosquitoes partially resistant of dengue virus. They are also trials with genetically modified male *A.aegypti* that after release into the wild mate with females, and offspring live through the larval stage but dies as pupae, before reaching sexuality (Whitehorn *et al.*, 2010).

There are ongoing programs working on a dengue vaccine to cover all four serotypes. One of the concerns is that a vaccine could increase the risk of severe disease through antibody-dependent enhancement (ADE). The ideal vaccine is safe,

effective after one or two injections covers all serotypes, does not, contribute to ADE. Is easily transported and stored and is both affordable and cost effective. As at 2012, a number of vaccines were undergoing testing. The most developed is based on a weakened combination of yellow fever virus and each of the four dengue serotypes. It is hoped that first products will be commercially available by 2015.

Apart from attempts to control the spread of the Aedes mosquito and work to develop a vaccine against dengue, there are ongoing efforts to develop antiviral drugs that would be used to treat attacks of dengue fever and prevent severe complications. Discovery of the structure of the viral proteins may aid the development of effective drugs. There are several plausible targets. The first approach is inhibition of the viral RNA-dependent, RNA polymerase (coding by NS5), which coding the viral genetic material, with nucleotide analogs. Secondly, it may be possible to develop specific inhibitors of viral protease (coded by NS3), which splices viral proteins. Finally, it may be possible to develop entry inhibitors, which stop the virus entering cells or inhibitors of the 5' capping process, which is required for viral replication (Whitehorn *et al.*,2010).

2.15 AEDES MOSQUITO

Aedes mosquito is the primary vector for dengue fever and yellow fever (Black *et al.*,2002). The species that serves as vector for dengue virus are *Aedes aegypti* and *A. albopictus*. Aedes mosquito *is* a small, dark mosquito with white lyre shaped markings and banded legs. They prefer to bite indoors and primarily bite humans. These mosquitoes can use natural locations or habitats (for example tree holes and plant axils) and artificial containers with water to lay their eggs. They lay eggs during the day in water containing organic material (e.g. decaying leaves, algae, etc.) in containers with wide openings and prefer dark colored containers located in the shade. About three days after feeding on blood, the mosquito lays her eggs inside a container just above the water line. Eggs are laid over a period of several days, are resistant to desiccation and can survive for periods of six or more months. When rain floods the eggs with water, the larvae hatch. Generally larvae feed upon small aquatic organisms, algae and particles of plant and animal material in water-filled containers. The entire immature or aquatic cycle (i.e., from egg to adult) can occur in as little as 7-8 days. The life span for adult mosquitoes is around three weeks. Egg production sites are within or in close proximity to households. *Aedes aegypti* do not remain alive through the winter in the egg stage in colder climates.

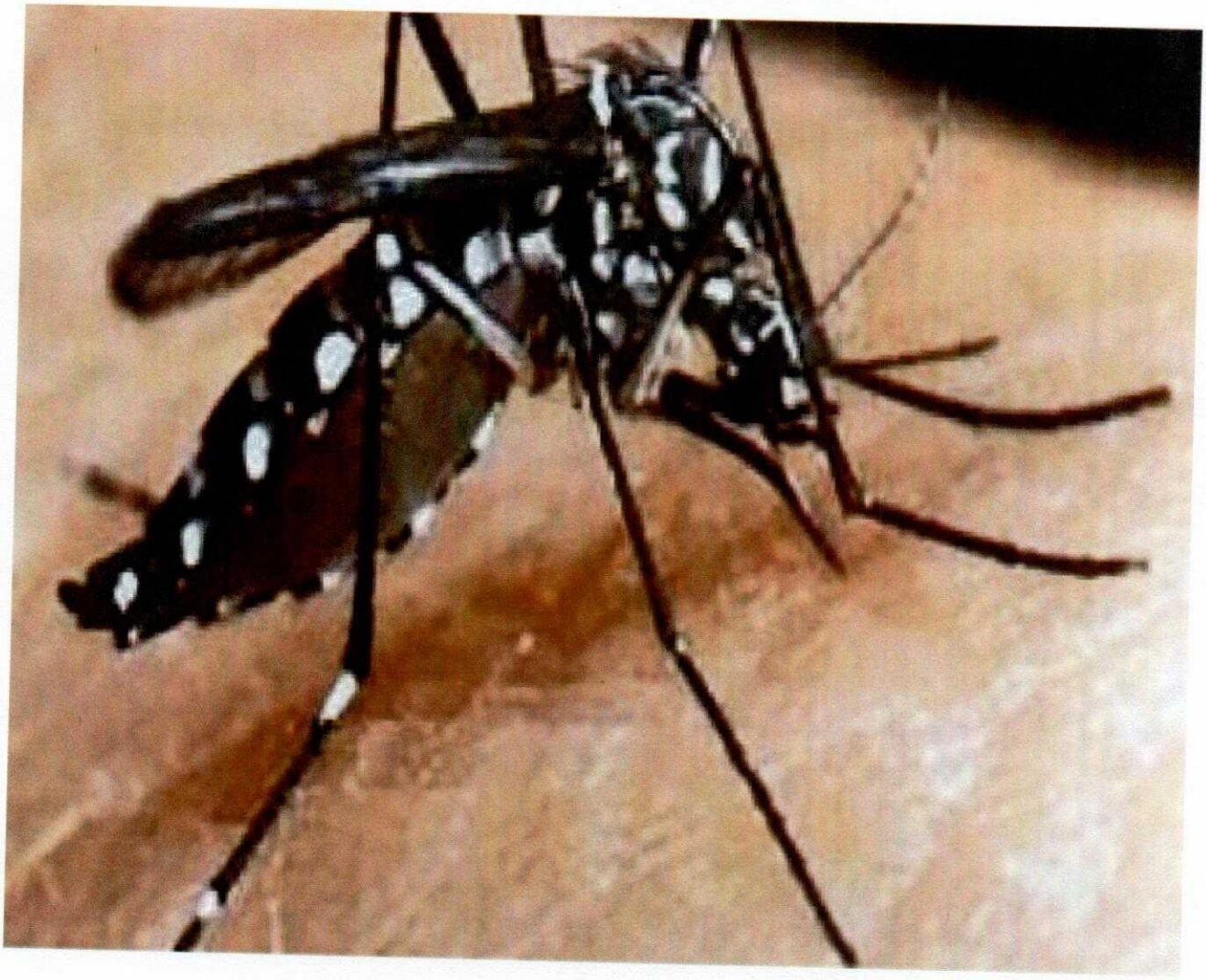

Figure 3: Diagram showing *Aedes aegypti*

Source: Wikipedia

2.16 GEOGRAPHICAL DISTRIBUTION OF AEDES MOSQUITO

Aedes mosquito is widespread throughout the world, including Africa, Argentina, Australia, Brazil, Caribbean islands, china, Cook Islands, Fiji, India, Japan, Malaysia, Morocco, New Caledonia, Papua New Guniea, Peru, Philippines, Portugal, Samoa, Seychelles, Surinam, Taiwan, Thailand, Vanuatu, and USA.

2.17 HABITS AND HABITATS

Aedes aegypti and A. *albopictus* is domestic container breeding species. It commonly breeds in artificial container including water drum (Chadee *et.al.*,2000), roof guttering, tyres, subterranean waters and refuse filled by rain. Aedes mosquito will also breed in natural container such tree holes and leaf axils of bromeliads (Forattini, 2000).

2.18 DETECTION OF DENGUE VIRUS IN AEDES MOSQUITO

The Dengue virus can be detected in individual or pooled mosquitoes by enzyme-linked immunosorbent assay (ELISA) and immunofluorescence assay (IFA) for viral antigens, by reverse transcription-polymerase chain reaction (RT-PCR) for viral RNA, and by isolation of infectious virus(Samuel *et al.*,2006).However, DENV surveillance in mosquito vectors using these diagnostic techniques can be prohibitively expensive, may require special reagents, equipment or laboratory

facilities or extensive training of personnel, and may be laborious and time-consuming. An ideal test method for DENV surveillance in vectors would be simple to perform, rapid, inexpensive, cost-efficient, sensitive and specific, and capable of detecting the pathogen under field-relevant conditions. For example, the triturated suspensions of large pools of mosquitoes, which are viscous and contain particulates and environmental contaminants, can complicate pathogen detection, especially by virus isolation and RT-PCR. In some circumstances, e.g., remote locations, mosquito traps may not be visited for extended periods of time, resulting in mosquito desiccation.

In addition, mosquitoes may be subjected to cycles of freezing and thawing during identification, pooling, processing, and assaying the samples. All of these field-relevant conditions can result in infectious virus inactivation and/or destruction of viral analytes.

The RT-PCR is widely used for detection of arboviruses, including DENV in field-collected or simulated field collected mosquito pools. The RT-PCR has been shown to detect one mosquito infected with Japanese encephalitis virus in a pool of 1,000 mosquitoes after 14 days of simulated tropical conditions. (Johansen *et al.*,2002)

Dengue virus RNA can be detected in mosquitoes captured over a period of 28 days on sticky lure traps using RT-PCR(Bangs *et al.*,2001),and nested PCR has been shown to detect DENV in one infected mosquito head in pools of up to 59 negative mosquito heads(Chow *et al.*,1998). Chikungunya virus RNA can be detected in *A.aegypti* mosquitoes stored at 28 °C for 12 weeks (Mavale *et al.*,2012).Although an excellent test, RT-PCR is expensive and requires trained personnel, specialized equipment, and laboratory facilities.

Antigen detection systems using in-house ELISAs can be used for arbovirus surveillance in mosquitoes. Antigen detection kits are commercially available to detect West Nile virus and Saint Louis encephalitis virus in mosquitoes (Burkhalter *et al.*,2006).

Recently, Tan and others showed that a commercially available ELISA kit designed to detect DENV non-structural protein 1 (NS1) in human serum also could be used to detect DENV NS1 in infected *A.aegypti* (Tan *et al.*,2011). Dengue virus NS1 antigen was detected in mosquitoes at 10 days after infection in the laboratory with DENV serotypes 1, 2, 3, or 4, as well as in field-collected DENV-infected mosquitoes. The test was as sensitive as real-time RT-PCR in detecting DENV-infected mosquitoes. (Tan *et al.*,2011).

2.19 AIM AND OBJECTIVES

- The aim and objective of this research is to detect dengue virus in Aedes mosquito circulating in Oye and environs.
- To establish a possible sylvatic transmission of dengue virus in the locality.
- To check for prevalence of dengue virus in Aedes mosquitoes.

CHAPTER THREE

MATERIALS AND METHODS

3.0 SAMPLE COLLECTION

Aedes aegypti mosquitoes were collected in Oye –Ekiti, Ekiti State, Nigeria through mosquito life traps and larva were collected from the stagnant water found around the vicinity. They were collected based on their unique way of identification, with their white lyre markings and black speckled legs. The mosquitoes were further identify by an Entomologist.

3.1 STORAGE OF MOSQUITO SAMPLE

After the mosquitoes have been introduced to insecticides, they got knocked down, so as to prevent them from decaying, silica gel was heated in the oven until colour changes was observed and a little quality was put into the eppendorf tube and was blocked with little cotton wools as to prevent the silica gel from touching the mosquito directly, with that the mosquito have been preserved to last for longer period of time.

3.2 MATERIALS AND EQUIPMENT

Mosquito net, Cotton wool, Eppendorf tube, Micropipette, Eppendorf tips, Micro centrifuge, Vortex, Refrigerator, Micro speed column tube, Water bath, Elusion buffer, Electrophoretic machine, PCR machine, PCR set up reagent, Gloves, Blender. Scoopin spoon, Transparent white buckets, Adult Aedes mosquito Aedes mosquito larva, Mosquito cage.

3.3 RNA EXTRACTION

Mosquitoes Sample were blended finely and were transferred into Eppendorf tubes and 250*ul* of suspension buffer was added into tubes, the samples were crushed and mixed using vortex mixer for 10seconds

Lyses buffer was added and incubate at 55^0C for 30minutes and centrifuge at 12000rpm for 5minutes.

A supernatant was added into a new centrifuge tube and 100*ul* of precipitate buffer was added and centrifuge at 14000rpm for 5minutes.

250*ul* of supernatant was collected into another centrifuge tube and 375*ul* of the binding buffer was then added and centrifuged for 30seconds at 1000rpm and the collection tubes were discarded and replaced with new one.

500*u*l of washed buffer was added, and centrifuged for 2minutes at 1000rpm, the collection tube were removed, and replaced with new ones.

Washed buffer were added again and then centrifuge for 2minutes at 1000rpm. The flow through were then placed in new collection tubes.

Finally, 100*u*l of elution buffer was added and samples were incubated at room temperature for 5minutes and centrifuge at 14000rpm for 1minutes and

RNA solution was obtained

3.4 PROCEDURE FOR POLYMERASE CHAIN REACTION

Polymerase chain reaction (PCR) is used for the amplification of RNA extracted from the sample.PCR procedure is as follow;

The following were added into PCR tubes;

10*u*l of PCR master mix,

2*u*l of RNA solution,

2*u*l of the reconstituted forward and reverse primer.

20*u*l nuclease-free water in other to maintain the final volume.

PCR tubes were placed into the PCR machine and carry out the RT-PCR

The PCR machine was programmed.

3.5 PROCEDURE FOR ELECTROPHORESIS

TBE (Tris Borate EDTA) buffer was used to prepare agarose gel and TBE buffer was prepared by adding 25ml of 25x TBE to 225ml of distilled water.

Agarose gel was prepared by adding 1.5g of agarose powder to 100ml of distilled TBE buffer and heat in other to melt the powder.

The mixture was allowed to cool and 0.5 of visual violet dye was added to it.

Caster was assembled and comb was inserted into the caster.

When the gel was ready, the comb was gently removed.

10*ul* of RNA template was mixed with a drop of loading dye.

The mixture were loaded inside the well of the gel and PCR range/ladder was loaded into the first well.

Electrophoresis tank was filled with diluted buffer, the tray was placed inside the electrophoresis tank and the buffer was allowed to enter the well of the machine. The machine was connected to the power source by connecting the red wire to the red port and the black wire to the black port.

The power source was then set to 100v for 30minutes.

3.6 RESULTS

After 30minutes of the electrophoresis, the agarose gel was observed and no band was visualized on the gel which implies that the sample tested were negative to the presence of Dengue Virus.

Table 1: Negative result of Dengue virus in Aedes mosquitoes caught in Oye Ekiti, Ekiti State, Nigeria.

AEDES MOSQUITO SAMPLES	RESULT DERIVED
I	NEGATIVE
II	NEGATIVE
III	NEGATIVE
IV	NEGATIVE
V	NEGATIVE
VI	NEGATIVE
VIII	NEGATIVE
IX	NEGATIVE
X	NEGATIVE

CHAPTER FOUR

4.0 DISCUSSION

Dengue virus has been known to cause sequel infection in individuals, if left untreated for long time. An RNA isolation method was required for the detection of dengue virus in infected mosquitoes without RNA degradation or PCR inhibition. The report methods for the isolation of RNA from mosquitoes requires the use of costly reagents to avoid inhibition. The mosquito samples collected were stored and they were intact during the process of the extraction of the gene.

Furthermore, the extraction method was used to recover viral RNA from infected mosquitoes frozen after natural death, suggesting that even mosquitoes that have died during field collection can still furnish valuable information about viral infection. Dengue has become a global problem since the Second World War and is endemic in more than 110 countries. Apart from eliminating the mosquitoes, work is ongoing on a vaccine, as well as medication targeted directly at the virus. Due to the lack of a vaccine or cure for dengue fever, the development of laboratory-based surveillance system is critical in other to provide early warning of dengue fever epidemic. Such information can enable preventive measures (e.g. mosquito control) and enhance preparedness on the part of physicians, hospitals and the public.

However, effective surveillance have access to appropriate information. For Aedes mosquitoes caught in Oye Ekiti, Ekiti State, Nigeria there is no dengue virus present in the sample mosquitoes.

4.1 CONCLUSION AND RECOMENDATION

The negative result shows that dengue fever may not be endemic in Oye-Ekiti, Ekiti State, Nigeria since dengue virus is not present in Aedes mosquitoes caught in Oye-Ekiti, Ekiti State, Nigeria.

Individual should therefore maintained personal and proper environment sanitation so as to avoid the infestation of pests, avoidance of self-medication or treatment, and in cases of disease only prescribed drug by health physicians should be used.

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