SEROPREVALENCE OF DENGUE VIRUS ANTIBODIES AMONG FEBRILE CHILDREN IN VARIOUS HOSPITALS IN BENIN CITY, EDO STATE, NIGERIA

A PROJECT PRESENTED

BY

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DEPARTMENT OF MEDICAL LABORATORY SCIENCE

SCHOOL OF BASIC MEDICAL SCIENCES

COLLEGE OF MEDICAL SCIENCE

UNIVERSITY OF BENIN

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PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, UNIVERSITY OF BENIN

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SUPERVISED BY

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CERTIFICATION

This is to certify that this project work was carried out by Orugbo Oteroghogho with Matriculation number BMS2001197, under the supervision of Dr. (Mrs.) Ifueko,M. Moses-Otutu and submitted to the Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Benin City in partial fulfillment of the requirement for the award of Bachelor of Medical Laboratory Science Degree.

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DEDICATION

This project is dedicated to God Almighty for his infinite grace and strength that he bestowed upon me during the project work and indeed throughout my life.

ACKNOWLEDGEMENTS

Above all, I thank God Almighty, who enabled me with perception, understanding and motivation to present this work after so many hurdles and obstacles. Without him all efforts would have wrought to nothing.

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ABSTRACT

Dengue fever is endemic in Nigeria and remains a significant cause of misdiagnosed febrile illnesses, particularly in children. Despite rising Aedes mosquito populations in urban centers, limited data exists on dengue prevalence among pediatric populations in Benin City. This study's aim was to determine the prevalence of dengue virus infection among febrile children attending various medical centers in Benin City, Nigeria. A hospital-based cross-sectional study was conducted among 160 febrile children aged 1 month to 10 years attending selected healthcare facilities in Benin City. Socio-demographic data were collected using structured questionnaires. Blood samples were collected from each participants through sterile aseptic techniques and dispensed into plain containers. The samples were spun at 3000rpm for 5 minutes to obtain the serum. The overall prevalence of dengue virus IgM antibodies was 4.4% (7/160) among the study population. IgG antibodies prevalence of 3.1% (5/160) while both IgM and IgG antibodies coinfection of 0.6% (1/160) was obtained among the study population. Seroprevalence was highest among the 3-4 years age group, followed by 1-2 years age group with the lowest prevalence found in <11 months age group. Gender and Age did not significantly influence the prevalence of dengue virus antibodies among the study population. Use of mosquito nets and water storage methods significantly influenced the prevalence of dengue virus seroprevalence among the study population though not statistically significant. This study reveals a notable prevalence of dengue virus infection among febrile children in Benin City, highlighting the need for improved diagnostic considerations and public health surveillance in pediatric fever management.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

In recent years, global health has seen several positive changes, such as a steady decrease in child deaths and malaria cases, as well as the near elimination of polio worldwide. However, many challenges remain, particularly with new infectious disease outbreaks and growing rates of certain neglected tropical diseases (Bill, 2014). Dengue fever is one of these diseases. It has become more widespread, both in terms of the number of countries where it is found and the risk of severe forms, such as dengue hemorrhagic fever. The number of dengue cases worldwide has increased 30 times over the past 50 years. In terms of dengue risk, Nigeria is classified as having sporadic or uncertain levels (CDC, 2025). Dengue fever is considered the most significant arboviral disease globally. It is caused by any of the four dengue virus serotypes (DENV 1, DENV 2, DENV 3, and DENV 4) (Chukwuma *et al.,* 2018). Dengue is a mosquito-borne illness caused by a flavivirus, which is a type of enveloped single-strand RNA virus belonging to the Flavivirus genus (Krishna *et al.,* 2024).

This virus is transmitted by a female Aedes mosquito, primarily the Aedes aegypti and Aedes albopictus species (Krishna *et al.,* 2024). Dengue fever does not spread easily to animals, meaning the main way it spreads is through the human-mosquito cycle. The transmission of DENV from a human to a mosquito requires several biological factors to be present at the same time and place. Under natural conditions, a susceptible mosquito can become infected with DENV only after it takes a blood meal from someone who has the virus in their blood (Carrington *et al.,* 2014). In nearly all Nigerian states, dengue fever is present and is one of the main causes of misdiagnosed fever-related illnesses (WHO, 2023).

Although dengue fever has historically been more common in urban areas, it is now found in both urban and rural regions. Due to a lack of public health priority, low public awareness, and healthcare professionals' limited understanding of its clinical symptoms, dengue is often underdiagnosed and misdiagnosed as other unclassified febrile illnesses in Nigeria (Nasir *et al.,* 2017). The actual prevalence of dengue fever in Nigeria is greatly underreported (Ayukekbong *et al.,* 2017). The symptoms of dengue fever include fever, headache, muscle and joint pain, and a skin rash that looks like measles. In some cases, the illness can progress to dengue hemorrhagic fever, which can cause bleeding, a drop in platelet count, and blood plasma leakage. In more severe cases, it can lead to dengue shock syndrome, where blood pressure drops dangerously low (Heilman *et al.,* 2014).

Fever is one of the most common symptoms in children's illnesses. In children under five years of age, a fever usually indicates systemic inflammation, typically caused by a virus, bacteria, parasite, or less commonly, a non-infectious cause (Herlihy *et al.,* 2016). National survey data was collected and analyzed from 42 Sub-Saharan African countries (excluding Botswana, Cabo Verde, Eritrea, and South Africa) to estimate 655.6 million episodes of fever in children under five in 2007, with 32 percent occurring in 11 outpatient units in the Democratic Republic of Congo, Ethiopia, and Nigeria (Herlihy *et al.,* 2016). Acute febrile diseases spread by mosquitoes cause high rates of illness and death among children in Sub-Saharan Africa (WHO, 2022). According to the World Health Organization, there were 247 million cases and 619,000 deaths from Plasmodium infections, mainly from P. falciparum and P. vivax, in 2021. About 96% of these deaths occurred in Africa, and 80% were among children under five years of age (WHO, 2015). Increased awareness and the use of artemisinin-based combination therapy have led to a significant decrease in malaria cases in recent years (Froeschl *et al.,* 2018). At the same time,

there has been an increase in cases of malaria-like illnesses caused by viral and bacterial pathogens, with dengue fever being one of them. Dengue fever is endemic in almost every state in Nigeria and is one of the main causes of misdiagnosed fever-related illnesses (WHO, 2023). This study aims to assess the burden of dengue virus among febrile children in Benin City, thereby providing valuable data for clinical diagnosis and public health surveillance.

1.2 JUSTIFICATION OF STUDY

Children are a particularly vulnerable group, often exposed to mosquito bites in poorly screened environments and lacking immunity due to limited prior exposure. Febrile illnesses are common reasons for pediatric hospital visits, but testing for dengue is rarely considered. Febrile illness in children is a major health issue in Nigeria. In most cases, the first thought when a fever is observed is malaria, and typhoid fever is considered next and may be treated without confirmation (Okoror *et al.,* 2021). However, many cases remain misdiagnosed because the symptoms of dengue, malaria, and typhoid overlap (Moses *et al.,* 2016).

Dengue virus infection is often not taken into consideration by doctors, as the disease is not considered endemic in the region, which can lead to serious consequences (Okoror *et al.,* 2021). The increasing emergence of dengue fever, especially in urban areas, is poorly tracked in pediatric cases. Without accurate data on the disease's prevalence, healthcare workers often overlook dengue, resulting in missed diagnoses and incorrect treatment. Previous studies have mostly focused on adults, outbreak scenarios, seroprevalence, or detection of dengue fever (Ayolabi *et al.,* 2019; Otu *et al.,* 2019). This study addresses the lack of data on dengue prevalence in routine pediatric fever cases and in Benin City. Despite the rising population of Aedes mosquitoes, there is no recent seroprevalence data for pediatric dengue in Benin City. This

study aims to assess the burden of dengue virus among febrile children in Benin City, providing valuable data for clinical diagnosis and public health surveillance in Nigeria as a whole.

1.3 AIM OF STUDY

The aim of this study was to determine the prevalence of dengue virus infection among febrile children attending various medical centers in Benin City, Nigeria.

1.4 SPECIFIC OBJECTIVES

The specific objectives of the study are;

1. to detect the presence of dengue virus infection using IgM/IgG-based test kits.
2. to assess demographic characteristics (age, sex, residence) associated with dengue virus positivity

1.5 RESEARCH QUESTION

1. What is the prevalence of dengue IgG and IgM antibodies among febrile children in Benin City?
2. Are there specific demographic or clinical patterns among children testing positive for these antibodies?

1.6 HYPOTHESES

1. There is no significant prevalence of dengue virus among febrile children in Benin City (null hypothesis).

   There is significant prevalence of dengue virus among febrile children in Benin City(alternate hypothesis).

2. The prevalence rate of dengue fever will be the same in children aged 6-10 years compared to 1-5 years (null hypothesis).

   The prevalence rate of dengue fever will be higher or lesser in children aged 6-10 years compared to 1-5 years (alternate hypothesis).

CHAPTER TWO

LITERATURE REVIEW

2.1 HISTORICAL BACKGROUND OF DENGUE VIRUS

Historically, it is unclear when dengue virus first appeared in human populations, mainly because the disease often does not show symptoms. Dengue fever was first mentioned in a Chinese medical book in 992 BC (Salles *et al.,* 2018). At that time, it was called 'water poison' and was connected to flying insects (Adesola *et al.,* 2024). Before the end of the 18th century, there were sporadic outbreaks of a disease that resembled dengue in Asia and the Americas. This leads people to believe that the virus likely spread across the tropics and subtropics during the 19th and 20th centuries. Brathwaite Dick and his team outlined the history of dengue outbreaks from 1600 to 2010, breaking it into four main periods: the introduction of dengue in the Americas (1600–1946), a plan to eliminate the Aedes aegypti mosquito (1947–1970), during which the mosquito was successfully removed in 18 countries, the reinfestation of the mosquito (1971–1999), which happened because the eradication plan failed (San Martin *et al.,* 2012).

In the Americas, a new outbreak happened around the time of several large global sporting events: the 2011 Pan-American Games in Guadalajara, Mexico; the 2013 Confederations Cup; the 2014 World Cup; and the 2016 Olympics, all held in Brazil (Salles *et al.,* 2018). This led to a rise in dengue-related deaths. According to the Pan-American Health Organisation (PAHO), as of 2025, there were 3,739,526 dengue cases, 5,929 severe cases, and 1,881 deaths in the Americas, with an average of 367 cases per 100,000 people (PAHO, 2025). Dengue fever has become a major global public health issue in recent times, especially in the Asia-Pacific and Americas-Caribbean regions. Its spread and impact in Africa are not yet well understood. Aedes species of mosquitoes are widely found across Africa and can carry the dengue virus. If their presence is combined with rapid population growth, unplanned urban development, and more

international travel, dengue infection is expected to spread widely across Africa. The main mosquito, *Aedes aegypti*, which plays a key role in the disease becoming a public health concern, spread out of Africa between the 15th and 19th centuries, in part due to the slave trade and increasing global connections, and is now found in many tropical and subtropical parts of the world (Simmons *et al.,* 2012). Although dengue outbreaks were described as early as the 17th century, the first reliable reports of such outbreaks were in 1779 and 1780, when diseases affected Asia, Africa, and North America. Outbreaks were rare until the 1940s. The transmission by Aedes mosquitoes was confirmed in 1906, and dengue fever was the second disease after yellow fever to be shown to be caused by a virus in 1907. In the last 50 years, many countries in Sub-Saharan Africa have reported dengue outbreaks, as shown in Figure 2.1. Although there have been rare cases of Dengue Hemorrhagic Fever (DHF) in some African countries, full outbreaks have not been recorded (Diallo *et al.,* 2022).

Dengue fever was noted in Africa in the late 19th and early 20th centuries. Epidemics took place in Zanzibar (1823, 1870) (Tamura *et al.,* 2022), Burkina Faso (1925), Egypt (1887, 1927), South Africa (1926–1927), and Senegal (1927–1928). The South African outbreak was confirmed through antibody testing in the mid-1950s, but the other outbreaks were not scientifically verified and might not have been dengue. From 1960 to 2010, 20 laboratory-confirmed dengue outbreaks were recorded in 15 African countries, mostly in eastern Africa (Izmirly *et al.,* 2020). Five large outbreaks documented nearly 300,000 cases in the Seychelles (1977–1979), Réunion Island (1977–1978), Djibouti (1992–1993), the Comoros (1992–1993), and Cape Verde (2009). The dengue virus was first found in Nigeria in the 1960s (Adesola *et al.,* 2024). As a result, all four dengue serotypes were found in Africa. Most outbreaks were linked to DENV-2, with DENV-1 being the next most common.

Figure 2.1 A Map Showing The Distribution Of Aedes Spp. And Dengue Virus In Africa (Were *et al.,* 2012).

2.2 TAXONOMY AND CLASSIFICATION OF DENGUE VIRUS

The dengue virus has a defined place in the biological classification system, helping scientists understand its relation to other viruses and its unique features.

Hierarchical Taxonomy:

Viruses/ Realm: Riboviria

Kingdom: Orthonavirae

Phylum: Kitrinoviricota

Class: Flasuviricetes

Order: Amarillovirales

Family: Flaviviridae

Genus: Orthoflavivirus

Species: Orthoflavivirus denguei

Virus Isolate: Dengue Virus

(Schoch *et al.,* 2020)

The Dengue virus has four (4) serotypes: DENV 1, DENV 2, DENV 3, and DENV 4.

DENV 1 has five (5) genotypes: I, II, III, IV, and V. Genotype V of DENV 1 is based on its geographical spread and includes: Asia, Africa, America, and the South Pacific (de Bruycker-Nogueira *et al.,* 2016).

DENV 2 has six (6) genotypes: Asian I, Asian II, Cosmopolitan/Brazil, American, American/Asian, and sylvatic (Waman *et al.,* 2016).

DENV 3 has five (5) genotypes: I, II, III, IV, and V. DENV 4 has four (4) genotypes: I, II, III, IV, and sylvatic.

2.3 VIROLOGY AND STRUCTURE OF DENGUE VIRUS

STRUCTURE OF DENGUE VIRUS

Dengue virus (DENV) is a positive-sense single-stranded RNA virus that belongs to the Flavivirus family. It has spherical-shaped virus particles with surface proteins arranged in an icosahedral pattern. The genome of DENV is approximately 11 kilobases long and is directly translated into host cells, producing both structural and non-structural proteins. The virus has a roughly spherical shape with a diameter of about 50 nanometres. The centre of the virus is the nucleocapsid, which consists of the viral RNA and C protein. The nucleocapsid is surrounded by a membrane called the viral envelope, which is a lipid bilayer sourced from the host. The viral envelope contains 180 copies of the E and M proteins, which span the lipid bilayer. These proteins form a protective outer layer that helps the virus enter human cells. The mature DENV virion has a smooth surface and a diameter of about 50 nanometres, whereas the immature virion has a diameter of about 60 nanometres and a spiky surface. The genome encodes three structural proteins: capsid (C, 100 amino acids), pre-membrane/membrane (prM/M, 75 amino acids), and envelope (E, 495 amino acids), along with seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). Structural proteins make up the DENV virion, while non-structural proteins are involved in RNA replication (Harapan *et al.,* 2020).

The C protein, which is about 12 kilodaltons, is a homodimeric protein composed of 100 amino acid residues, with 26 being basic and three being acidic. The C protein plays a key role in the production of the nucleocapsid during the early stages of DENV virion assembly (Byk and Gammarnik, 2016). The M protein is necessary for the organisation and maturation of DENV particles. The E protein consists of three domains, with the third domain playing an important role in receptor binding (Dwivedi *et al.,* 2017). The E protein is essential for the interaction and fusion of the virus with the host cell membrane (Dwivedi *et al.,* 2017).

NS1 is a 45 kilodalton N-linked glycoprotein that is involved in the RNA replication complex. It is initially produced as a monomer and is processed in the endoplasmic reticulum and the trans-Golgi network before being secreted as a hexameric lipoprotein particle into the extracellular space and blood. NS1 is used as a target for diagnostic tests such as enzyme-linked immunosorbent assays (ELISA) and rapid immunochromographic tests(Zhang *et al.,* 2014). Single-NS1-based testing is a useful diagnostic method for screening and confirming DENV infection. NS2A is a 22 kilodalton protein involved in the replication complex. NS3, which is 618 amino acids long, performs several enzymatic functions, including chymotrypsin-like serine protease activity, RNA helicase activity, and RTPase/NTPase activity. NS4A and NS4B, which weigh 16 and 27 kilodaltons respectively, are integral membrane proteins that cause changes in the membrane necessary for DENV replication (Harapan *et al.,* 2020).

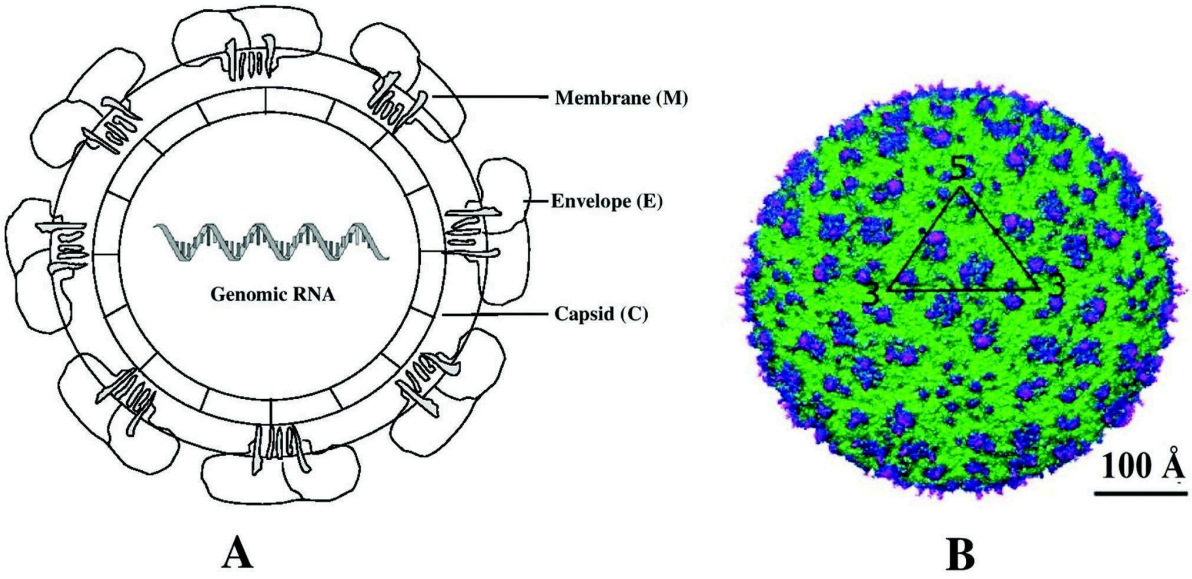

Figure 2.2 (A) depicts an enveloped and spherical DENV virion with different structural proteins, and (B) shows the cryo-electron microscopic structure of the DENV-4 virus.

The black triangle indicates the icosahedral asymmetric unit. The E protein dimers are shown in blue, with three E proteins in one asymmetric unit. The scale bar represents 100 Å (Kostyuchenko *et al.,* 2014).

GENOME OF DENGUE VIRUS

The DENV genome has low translational fidelity and a high mutation rate (approximately 10-3 to 10-5 per nucleotide per round of replication) (Harapan *et al.,* 2020). The genome, which is around 11 kilobases long, is a positive-sense single-stranded RNA (ssRNA) that consists of a single long open reading frame (ORF) flanked by two untranslated regions (5'-UTR and 3'-UTR) (Roy and Bhattacharjee, 2021). It has a type 1 cap structure (m7GpppAmpN2) at the 5'-UTR but no poly(A) tail at the 3'-UTR (Harapan *et al.,* 2020). The 5'-UTR, which is 95 to 101 nucleotides long, includes six elements: stem-loop A (SLA), stem-loop B (SLB), 5'-upstream AUG region (5'-UAR), 5'-downstream AUG region (5'-DAR), C-coding region hairpin (cHP), and 5'-cyclization sequence (5'-CS). The final three components are located within the C protein-coding region. SLA functions as a promoter for the viral RNA-dependent RNA polymerase (RdRp, also known as NS5). The interaction between NS5 and SLA is essential for viral replication. SLB includes regions involved in long-range RNA-RNA interactions necessary for genome replication (Robinson *et al.,* 2025). Genome cyclisation involves both the 5' and 3' UARs. The 5'-DAR is involved in both RNA replication and DENV circularisation. The cHP, which is 14 nucleotides long, initiates translation from the C-start codon. The 5'-CS facilitates RNA-RNA contact between the 5' and 3' ends of the viral genome and is therefore important for genome cyclisation.

The ORF, which encodes both structural and non-structural proteins, is translated into a polyprotein, which is then processed co- and post-translationally by cellular and viral proteases to produce 10 mature viral proteins. The N-terminal part of the ORF encodes the three structural proteins (C, prM/M, and E), which are followed by the seven NS proteins. The 3'-UTR is

approximately 450 nucleotides long and plays a vital role in DENV replication, regulating viral proliferation and RNA synthesis in mammalian cells (Harapan *et al.,* 2020).

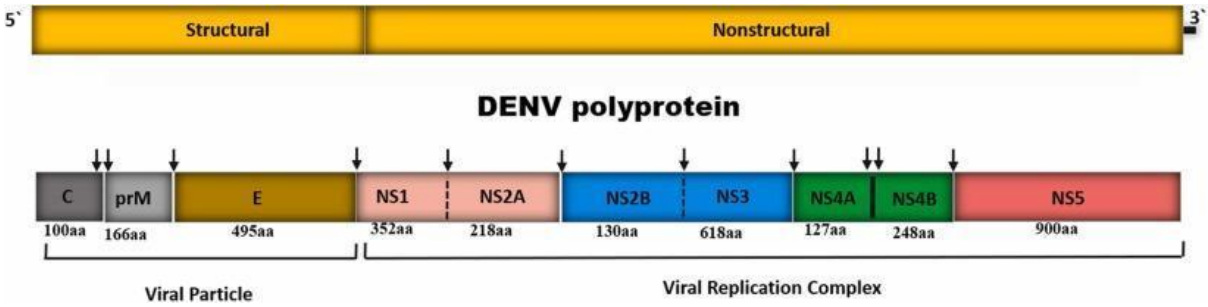

Figure 2.3 Structure Of The Genome Of Dengue Virus (Mushaq *et al.,* 2023).

LIFE CYCLE OF DENGUE VIRUS

The life cycle of DENV typically involves transmission to humans through infected mosquitoes such as *Aedes aegypti* or *Aedes albopictus (*Ferreira-de-Lima *et al.,* 2018).

On the first day of infection, the virus targets dendritic cells (DCs) and macrophages. According to previous reviews, the DENV life cycle includes processes like viral entry and attachment, endosome membrane fusion, nucleocapsid release, protein synthesis and processing, RNA replication, nucleocapsid formation, viral assembly, viral maturation, and the release of mature DENV particles (Campos *et al.,* 2018; Kato and Hishiki, 2016).

The life cycle also involves mechanisms such as viral entry and attachment, endosome membrane fusion, nucleocapsid release, protein synthesis and processing, RNA replication, nucleocapsid production, viral assembly, viral maturation, and the eventual release of mature DENV particles. DENV E protein interacts with components in target cells, such as DC-SIGN, the mannose receptor, heparan sulphate, and other molecules, to adhere to the host cell (Cruz-Oliveira *et al.,* 2015). Once attached, DENV enters the cell through a process called clathrin-mediated endocytosis. The acidic environment inside the endosome allows the virus to fuse with the endosomal membrane (Kato and Hishiki, 2016). In this acidic setting, the viral RNA is released from the nucleocapsid into the cytoplasm where it undergoes processing and replication (Campos *et al.,* 2018). Initially, the RNA acts as a message to produce viral proteins.

The replication of the RNA occurs in an intracellular membrane structure known as the replication complex. This complex includes viral RNA, viral proteins, and host factors (Teo and Chu, 2014). It forms on the endoplasmic reticulum (ER) membrane and helps protect the replication process from the host's immune system (Pierson and Diamond, 2020). As the nucleocapsid is formed, the virus starts to assemble in the ER lumen as an immature DENV

particle (Campos *et al.,* 2018). During the transport through the trans-Golgi network (TGN), the virus matures as the prM/M protein is cleaved by furin-like proteases into the M protein. This cleavage is necessary to release the mature and infectious virus. The prM/M protein prevents premature fusion of the viral membrane with the host cell before the virus is released by wrapping around the hydrophobic fusion loop of the E protein. Eventually, the prM/M protein separates from the virus when the mature DENV is released into the extracellular environment (Kok *et al.,* 2023).

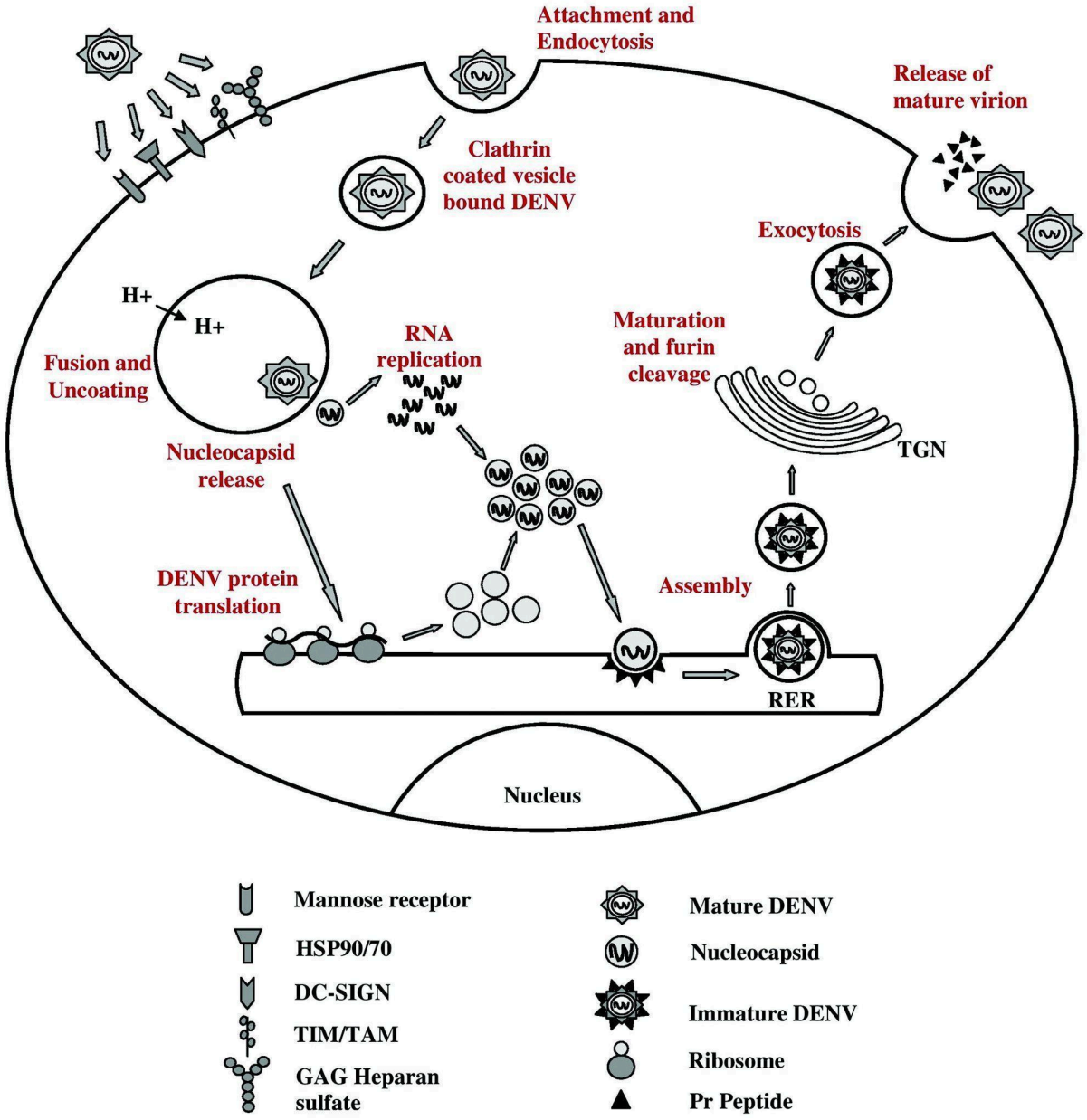

Figure 2.4 Life Cycle Of Dengue Virus (Roy and Bhattacharjee 2021).

Table 2.1 Table showing the different types of protein found in Dengue virus, its function and structure

Protein	Structure	Function
C	A 100 amino acid (aa) residue, homodimeric protein with four α-helical regions and an intrinsically disordered N-terminal domain.	C is involved in genome encapsidation.
prM/M	prM is a 166 aa residue protein, while M consists of 75 aa residue	prM/M functions as a cap-like structure that protects the fusion peptide E from undergoing premature fusion before virus release
E	AA 493–495 aa residue (53 kDa) class II N-glycosylated dimeric membrane fusion protein. In mature DENV, E is found as 90 homodimers that lie flat against the DENV surface forming a 'smooth' protein shell. Each of the monomer subunits is composed of three distinct domains (I, II and III).	E mediates virus binding and fusion to host cell membrane (domain III) and is responsible for the determination of host range, tropism and virulence (domain III).
NS1	A 46 kDa dimeric glycosyl-phosphatidylinositol (GPI) anchored protein that	NS1 is involved in the viral RNA replication complex as well as in viral defense through the

	exists in both intra- and extracellular forms.	inhibition of complement activation.
NS2A	A 218 aa residue protein, 22 kDa.	NS2A is involved in the coordination of change between RNA packaging and replication and antagonism of interferon (IFN).
NS2B	A 130 aa residue (14 kDa) protein is a membrane-associated protein.	NS2B associates with NS3 to form the DENV protease complex and serves as a cofactor in the structural activation of the DENV serine protease of NS3. NS3
NS3	A 618 aa (70 kDa) protein. The protease domain is N-terminal (residues 1–180) while the helicase domain is located at residues 180–618	NS3 is multifunctional protein with chymotrypsin-like serine protease, RNA helicase, and RNA triphosphatase (RTP/NTPase) enzyme activity, NS3 is involved in cleaving the DENV polyprotein as well as RNA replication.
NS4A and NS4B	NS4A and NS4B are small hydrophobic proteins that consist 150 aa (16kDa) and 245–249 aa (27kDa), respectively. Both of them are integral membrane proteins	NS4A induces membrane alterations that are important for virus replication. NS4B assists viral RNA replication through its direct interaction with NS3 and blocks IFN-induced signal

		transduction.
NS5	A 900 aa residue (104 kDa) protein is the most conserved DENV protein. The methyltransferase domain is located at residues 1–269 while the RNA-dependent RNA polymerase is located at residues 270–900	NS5 is a bifunctional enzyme with a methyltransferase and RNA-dependent RNA polymerase activity

(Harapan *et al.,* 2020)

2.4 CLINICAL FEATURES OF DENGUE FEVER

Dengue fever is a systemic and dynamic disease that manifests in a wide range of severity. In general, after an incubation period, the sickness is divided into three stages, namely:

1. Febrile stage
2. Critical stage
3. Recovery stage.

During the febrile phase, patients usually experience a sudden high fever that lasts between 2 to 7 days. This is commonly accompanied by symptoms such as facial redness, skin redness, body aches, muscle pain, joint pain, headaches, severe pain around the eyes, loss of appetite, nausea, vomiting, sore throat, red throat, and conjunctivitis. In the early stages, these symptoms are difficult to distinguish from those of other non-dengue fevers, and severe and non-severe dengue cases are often indistinguishable (Ng Dh *et al.,* 2016). The critical phase, or second phase, typically occurs around the time when the fever starts to subside, usually between days 3 and 7.

Defervescence is a temporary condition that lasts about 48 hours and is associated with an increased risk of capillary leakage and bleeding. At this stage, individuals without increased capillary permeability may improve, while those with increased capillary permeability might worsen due to plasma leakage, which can lead to life-threatening situations. If not treated, the critical phase can progress to shock, organ failure, disseminated intravascular coagulation, or internal bleeding. If shock persists, it can damage organs, cause metabolic acidosis, and result in widespread blood clotting, which can lead to serious bleeding and hemorrhage. Atypical clinical features of dengue may also develop without the obvious presence of plasma leakage or shock, including encephalitis, myocarditis, hepatitis, pancreatitis, retinitis, and acute respiratory distress syndrome. In this phase, two possible outcomes are either death or progression to the recovery

phase if the patient survives the first 24–48-hour critical phase. Some factors are associated with high dengue mortality (Harapan *et al.,* 2020).

The recovery phase involves the gradual reabsorption of extravascular fluid over 2 to 3 days. During this period, patients often exhibit bradycardia (Schaefer *et al.,* 2024). Persistent symptoms include headaches, retro-ocular pain, sleeplessness, alopecia, myalgia, arthralgia, asthenia, anorexia, dizziness, nausea, vomiting, and itching, which are linked to changes in specific immunological indices. Furthermore, a study indicated that neurological problems such as encephalopathy, myelopathy, myositis, and peripheral neuropathy are common after dengue infection (Harapan *et al.,* 2020).

CLINICAL SYMPTOMS FOR DENGUE INFECTION

Dengue virus causes a range of diseases ranging from Dengue fever, Dengue hemorrhagic fever e.t.c. The table below shows these dengue infections, their symptoms and duration for manifestation.

Table 2.2 Table showing the different types of infection caused by Dengue virus, their duration and symptoms

Dengue Infection	Symptoms	Duration
Dengue Fever (DF)	● "Flu-like" syndrome ● Retro-orbital pain ● Fever ● Rash ● Intense headache ● Intense joint and muscle pain ● Nausea	2 to 7 days
Dengue Hemorrhagic Fever (DHF)	● Plasma leakage ● Pleural effusion, bleeding ● Thrombocytopenia with <100,000 platelets/μL ● Raise in hematocrit levels ● Restlessness ● Abdominal pain ● Vomiting ● Sudden drop in temperature	After 3 to 5 days of fever
Dengue Shock Syndrome (DSS)	● Temperature reaches 37.5 °C–38 °C ● Hypotension ● Decrease in platelet count leads to leakage of plasma subsequent shock ● Fluid accumulation with respiratory distress ● Critical bleeding ● Organ impairment ● Cardiorespiratory failure and cardiac arrest	After 3 to 5 days of fever

(Wang *et al.,* 2020)

2.5 IMMUNE RESPONSE OF DENGUE FEVER

When the dengue virus infects the human host, both the innate (interferons, complement system, etc.) and adaptive immune systems (immunoglobulins, cytotoxic T-cells) are activated to neutralise the virus (Sinha *et al.,* 2024). The innate immune system quickly recognises and responds to DENV, but it does not produce a long-term or targeted response. The innate immune response activates the complement system, which assists antibodies and leukocytes in removing DENV. However, the adaptive immune system is more specialised, involving both cellular and humoral components. Both innate and adaptive immune responses to DENV infection help to resolve the illness and play critical roles in preventing reinfection (Harapan *et al.,* 2020). However, these responses may contribute to the enhancement of illness severity, resulting in severe dengue.

Innate Immunity

The immune system is made up of cellular and molecular organisations that perform specialised roles in the defence against foreign invaders and is essential for maintaining body homeostasis (Murphy and Weaver, 2016). Langerhans cells, cutaneous cells, and interstitial dendritic cells are the first targets of DENV infection (Harapan *et al.,* 2020). Other cells that may be targeted by DENV infection include monocytes, lymphocytes, Kupffer cells, alveolar macrophages, and endothelial cells. Upon DENV inoculation, host pattern recognition receptors (PRRs) such as endosomal Toll-like receptors (TLRs), retinoic acid inducible gene I (RIG-I), and melanoma differentiation-associated gene 5 (MDA5) are responsible for sensing or recognising DENV antigens (Harapan *et al.,* 2020). PPR molecules activate transcriptional factors IRF and NF-κB, leading to the generation of IFN-α/β and inflammatory cytokines (Garcia *et al.,* 2017). PPR molecules activate transcriptional factors IRF and NF-κB, leading to the generation of IFN-α/β

and inflammatory cytokines. This will stimulate dendritic cells, resulting in an antiviral response. In vitro investigations indicate that culture supernatants from DENV-infected cells contain a high quantity of cytokines. An in vivo investigation demonstrated a significant amount of cytokines such as TNF-α, IL-1β, IL-6, and IL-10 in serum of DENV-infected mice (Martinez-Moreno *et al.,* 2020). Severe dengue patients exhibit elevated plasma levels of IL-1β, IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-13, IL-18, TGF-1β, TNF-α, and IFN-γ. These cytokines and chemokines can play important protective or harmful functions during DENV infection (Harapan *et al.,* 2020).

This "storm" of inflammatory cytokines and other mediators is thought to operate on the endothelium and disrupt normal fluid barrier processes, resulting in increased plasma leakage (Harapan *et al.,* 2020). IFN-γ is a key cytokine generated during DENV infection. This cytokine controls DENV replication and infection resistance by regulating nitric oxide generation (Bhatt *et al.,* 2021). Increased IFN-γ levels protect against fever and high viremia, leading to improved survival rates in DHF patients (Qin *et al.,* 2025). In contrast, other proinflammatory cytokines appear to have a pathogenic effect. Increased TNF-α levels have been linked to worsening dengue symptoms and thrombocytopenia (Meena *et al.,* 2020). TNF-α has been linked to increased endothelial cell permeability in vitro, as well as apoptosis and haemorrhage in a mouse model. Another cytokine, IL-10, is linked to platelet degradation and may influence coagulation activation in DENV-infected patients (Harapan *et al.,* 2020). The macrophage migration inhibitory factor (MIF) also appears to play a negative impact in DENV infection. According to studies, the concentration of MIF is higher in severe dengue cases and correlates favourably with the severity of DENV infection. Other mediators include soluble substances such as monocyte chemoattractant protein 1 (MCP-1) and soluble vascular cell adhesion molecule 1 (VCAM-1) (Harapan *et al.,* 2020). During DENV infection, PRR activation also regulates the generation of

chemokines, which have both protective and pathogenic consequences. A study found that CXCL10 synthesis and CXCR3 activation boosted host resistance because they compete with DENV for cellular receptors and hence reduce DENV replication. Other chemokines, CCL2 and CCL5, have been linked to hypotension, thrombocytopenia, hemorrhagic shock, and hepatic dysfunction (Garcia *et al.,* 2017).

2.6 RISK FACTORS ASSOCIATED WITH DENGUE INFECTION

Dengue virus infection is impacted by a number of epidemiological, environmental, demographic, and behavioral factors that promote transmission and vulnerability. Understanding these risk factors is crucial for identifying susceptible populations and developing effective prevention tactics (Alqassim, 2024).

EPIDEMIOLOGICAL FACTOR:

Epidemiological factors that influence dengue infection include disease incidence patterns, transmission dynamics, and host-vector interactions within a community. These factors influence not only who becomes infected, but also the chance of an outbreak (Ardat *et al.,* 2024).

1. Previous dengue infection: Secondary infection with a different serotype raises the risk of severe disease due to antibody-dependent enhancement (Grange *et al.,* 2014).
2. Migration and travel spread novel dengue serotypes into non-endemic or partially immune populations, encouraging outbreaks (Je *et al.,* 2016).

ENVIRONMENTAL FACTOR:

Aedes aegypti, the principal vector of dengue, flourishes in tropical and subtropical regions (Das *et al.,* 2018). The following factors contribute to favourable breeding conditions:

1. Stagnant water and poor trash management: Containers, discarded tires, and water storage tanks make ideal breeding grounds for mosquitoes (Krystosik *et al.,* 2020).
2. Urbanisation and overcrowding: Greater population density in cities promotes mosquito-human contact, increasing transmission rates (Das *et al.,* 2018).
3. Climate change and rainfall patterns: Heavy rainfall, humidity, and higher temperatures encourage mosquito proliferation and shorten viral incubation time within the vector (Chandra and Mukherjee, 2022).

DEMOGRAPHIC FACTORS:

Certain groups are more prone to dengue infection and severe illness, these groups are determined via:

1. Age: Children are more susceptible to clinical dengue and serious sequelae such dengue hemorrhagic fever (Verhagen and de Groot, 2014).
2. Sex: Some studies imply that males have a larger infection rate for dengue due to more outside exposure, while severe symptoms are frequently more common in females.
3. Socioeconomic status: Populations with limited access to clean water, proper housing, and healthcare facilities are more vulnerable (Mulligan *et al.,* 2015).

BEHAVIORAL RISK FACTOR:

Individual and community habits have a substantial influence on the risk of dengue infection.

1. Water storage practices: Uncovered containers promote mosquito breeding (Das *et al.,* 2018).
2. Protective measures: The absence of mosquito nets, insect repellents, and window screens increases exposure (Jasman *et al.,* 2024).

3. Outdoor activities: Children and adults who spend time outdoors during peak mosquito bite hours (early morning and late afternoon) are at a higher risk

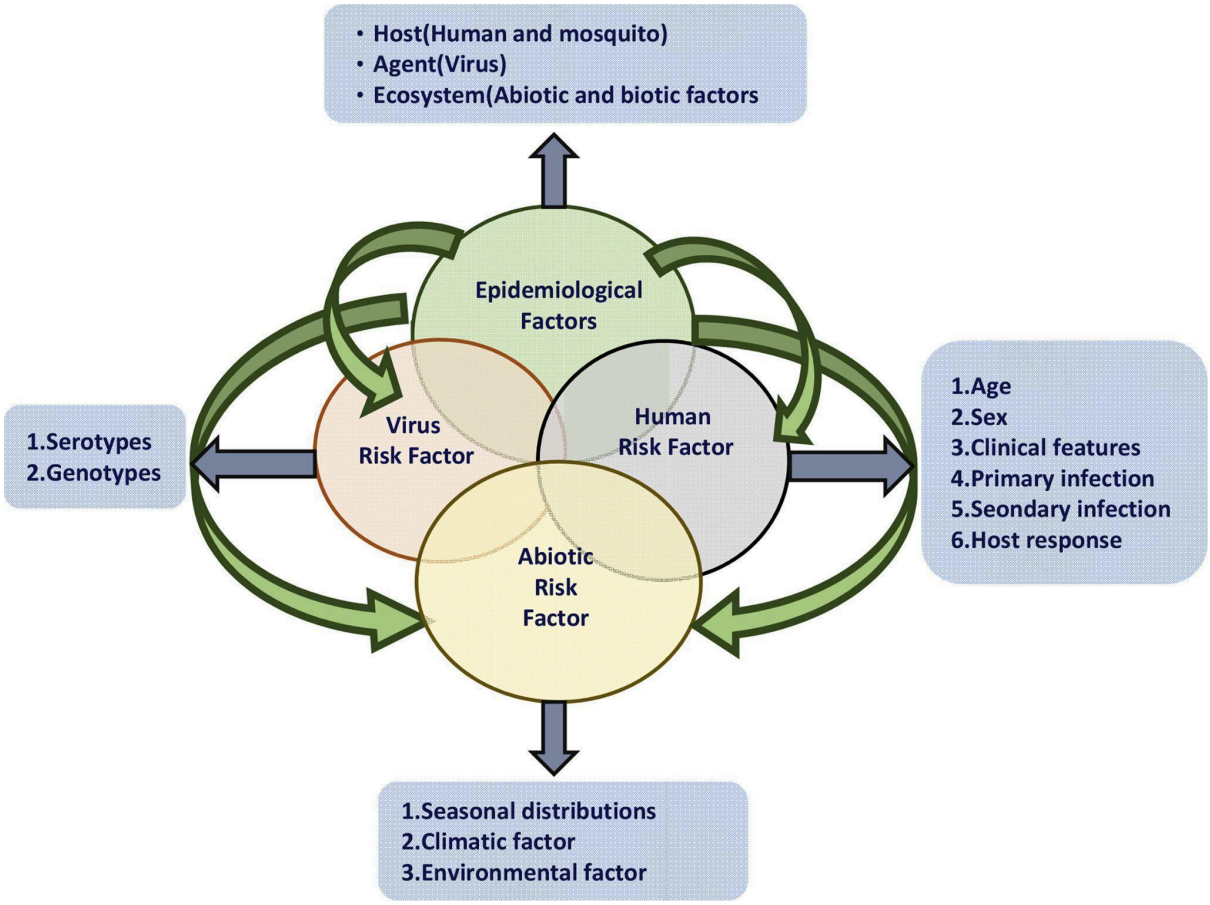

Figure 2.5 The Risks Factors Including Viral Factors, Epidemiological Factors, Human Factors And Abiotic Factors (Sinha *et al.,* 2024).

2.7 LABORATORY DIAGNOSIS OF DENGUE FEVER

Dengue diagnosis based solely on clinical symptoms is problematic due to the large range of non-specific symptoms observed during febrile sickness (Selvaraj *et al.,* 2025). Specific and sensitive diagnostic methods are available at various stages of illness. During early infection (<5 days), dengue can be detected using virus isolation, RNA detection (NAAT), or antigen detection (e.g., NS1). Following this period (>5 days after infection), DENV RNA and antigens may be undetectable, as viremia has reduced and antibody responses have mounted (Harapan *et al.,* 2020). At this time, specific antibody identification via serological methods (IgM or IgG detection) is suitable. The NS1 antigen can be detectable in certain patients for several days after defervescence (Harapan *et al.,* 2020).

Laboratory diagnosis of Dengue fever can be done via

1. Virus Isolation:

    Virus isolation is highly specific and can help confirm a DENV diagnosis. DENV can be obtained by inoculating clinical specimens onto cell lines such as the mosquito cell line C6/36 (Ae. albopictus) or mammalian cell lines Vero (African green monkey kidney), LLCMK2 (Monkey Rhesus kidney), and BHK21 (baby hamster kidney) (Kok *et al.,* 2023). Clinical specimens utilised for virus isolation can include whole blood, serum, plasma, or homogenised tissue (most commonly in fatal cases). Following inoculation and an incubation period, a confirmation assay such as immunofluorescence or reverse transcriptase polymerase chain reaction (RT-PCR) is necessary (Parkash and Hanim, 2015).

There are several practical limitations to virus isolation: (a) it takes a long time, needing at least 7 days for incubation and confirmation tests; (b) it requires well-equipped laboratories with skilled staff; (c) samples can only be collected during the early, acute stage of infection; and (d) low levels of DENV virus in the blood are not suitable for culturing (Parkash and Hanim, 2015).

2. Nucleic Acid Amplification:

Nucleic Acid Amplification techniques can diagnose dengue during the acute phase (<5 days) and detect DENV RNA in clinical specimens within 24-48 hours of infection. Techniques include RT-PCR, real-time RT-PCR, and isothermal amplification. RT-PCR can be performed using the nested RT-PCR method, one-step multiplex RT-PCR (a combination of four serotype-specific oligonucleotide primers in a single reaction tube), or the one-step pan-flaviviruses quantitative RT-PCR test (Vina-Rodriguez *et al.,* 2017). The sensitivity of RT-PCR procedures ranges from 80% to 100%, depending on the genomic area targeted by primers, the strategy used to amplify or detect PCR products, and the method used for serotyping. Multiplex real-time RT-PCR assays are quicker and can assess virus titers in clinical samples. However, this test involves costly equipment and reagents, and it must be conducted by skilled technicians. One-step pan-flaviviruses quantitative RT-PCR assay have equivalent characteristics compared to species-specific RT-PCR assay (Vina-Rodriguez *et al.,* 2017).

3. Serologic Testing:

Serological techniques such as hemagglutination inhibition (HI) assay and ELISA to identify IgM and IgG are more extensively used to diagnose dengue in developing nations, as they are straightforward to run, relatively affordable and the specimens required are stable at room temperature. The HI assay works by checking if the E protein can cause red blood cells to

clump together. Anti-DENV antibodies in sera limit agglutination, and the amount of this inhibition is evaluated using the HI assay . The HI assay has some disadvantages that make it unworkable, such as: (a) each serotype has a distinct optimum pH of RBCs, hence numerous pH buffers are required; (b) It cannot distinguish infections between DENV and other related Flaviviruses or between immunoglobulin isotypes (IgM vs. IgG); and (c) it may necessitate chemical and thermal pre-treatment to eliminate nonspecific inhibitors in clinical specimens (Parkash and Hanim, 2015). As a result, this technique has been mainly supplanted by ELISA-based approaches for detecting dengue-specific IgM and IgG. The sensitivity and specificity of IgM detection ELISA for sera obtained five days or longer after fever onset are approximately 90% and 98%, respectively. A recent study found that the quality of the antigen utilised has a significant impact on the sensitivity and specificity of IgM-based assays, which might vary substantially amongst commercially available kits (Haraban *et al.,* 2020).

4. Detection of Antigen:

New ELISA and fast immunochromographic (IC) tests targeting NS1 have been shown to identify primary and secondary DENV infection up to 9 days after disease start. A meta-analysis of 30 research from diverse nations discovered that the Panbio NS1 ELISA kit had 66% sensitivity and 99% specificity, whereas the Platelia NS1 ELISA kit had 74% sensitivity and 99% specificity. Another meta-analysis indicated that the IC technique based on NS1 antigen detection was marginally more sensitive than ELISA (71% vs. 67%) (Zhang *et al.,* 2014). In general, NS1-based tests are useful for detecting and confirming DENV infection (Zhang *et al.,* 2014).

2.8 PREVENTION AND TREATMENT OF DENGUE FEVER

Dengue fever has no specific antiviral treatment; therefore, prevention and control efforts focus on vector management, personal protection, community participation, and health system strengthening. Effective strategies must target both the mosquito vector (*Aedes aegypti*) and the human population at risk.

Ways to prevent Dengue fever is as follows:

1. Prevent Mosquito Bites:

When going outside homes, long fitting clothes should be worn. Also, the use of mosquito nets and mosquito repellents at home should be practiced (CDC, 2025).

2. Plan for Travel:

Before planning for any trip or vacation plans, examine country-specific travel advisories, health alerts, and warnings. Also, include paracetamol (also known as paracetamol) in your first-aid kit. These drugs can be used to control fever and bodily ache in the event you have dengue (CDC, 2025).

TREATMENT OF DENGUE

Dengue has no specific therapy, but pain can be treated with medications like paracetamol. Nonsteroidal anti-inflammatory drugs, such as ibuprofen and aspirin, should be avoided since they increase the risk of bleeding. Hospitalisation is frequently required for patients suffering from severe dengue (WHO, 2025).

2.9 VECTOR OF DENGUE VIRUS

Aedes mosquitoes are vectors of the dengue virus. During outbreaks, DENV spreads in the urban environment between humans and the mosquito species *Aedes aegypti* and *Aedes albopictus.* Aedes vectors have been detected breeding indoors and outdoors in human-associated water storage containers (clay jars, drums, jerrycans, cement tanks, etc.), discarded containers, old tires, flower pots, miscellaneous, etc. (Diallo *et al.,* 2018). These Aedes are primarily anthropophilic, although they have also been observed to eat other species (Diouf *et al.,* 2021) . *Aedes aegypti* is considered primarily endophilic, endophagic, and daytime feeder, but it was collected feeding and resting outdoors within used tires, bricks, and scrap metals, indicating that it can also transmit viruses outside (Diallo *et al.,* 2020).

Alternatively, *Aedes albopictus* is thought to be a more opportunistic and outside feeder. Climatically favourable locations for *Aedes aegypti* linked to dengue incidence are expected to rise in the future (Sintayehu *et al.,* 2020). Temperature and precipitation (which provide breeding locations and accelerate egg hatching) were discovered to be major climatic elements influencing *Aedes aegypti* development and dispersal.

DENV was linked to *Aedes aegypti* in an urban cycle in Senegal, Nigeria, Burkina Faso, Cabo Verde, Tanzania, Kenya, and Sudan during epidemic investigations and/or routine entomological studies (Diallo *et al.,* 2022).

2.10 FEBRILE CHILDREN

Fever is one of the most prevalent symptoms of childhood diseases. Fever in children under the age of five indicates systemic inflammation, which is often caused by a viral, bacterial, parasitic, or, less commonly, noninfectious aetiology (Herlihy *et al.,* 2016). Normal body temperature

varies by person and during the day. Preschoolers have the highest normal body temperature. Several studies have shown that peak temperature occurs in the afternoon and is highest between the ages of 18 and 24 months, when many typical healthy youngsters have a temperature of up to 38.3° C (101° F) (Consolini *et al.,* 2025).

Fever is typically defined as a core body temperature (rectal) of at least 38.0°C (100.4°F). The significance of fever is determined by the clinical context rather than the peak temperature; some minor infections have high fevers, while others induce only slight temperature elevations.

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY DESIGN

A hospital-based cross-sectional design was employed. The methodology was chosen because it enables the measurement of dengue virus antibody prevalence among febrile children at a specific time point, without the requirement for long-term follow-up.

In this study, data was collected from children who came with febrile illness at several hospitals in Benin City, Edo State. From each participants a structured questionnaire was used to obtain demographic and clinical information.

3.2 STUDY AREA

This research was carried out in selected Health facilities within Benin Metropolis. Benin city is a commercial town that is largely populated with an average population of 3,233,366 (Moses-Otutu *et al.,* 2023), it is located between latitude 6° 44'N and 6°21'N and longitude 5°35'E and 5°44'E (Iyalomhe and Cirella, 2018). This town has four local governments areas Egor, Uselu, Ovia North-East. The official languages spoken are Benin, Esan and English. The populace are predominantly traders, commercial workers, house wives, students and health workers.

3.3 SAMPLE POPULATION

The sample population included children aged 1 months to 10 years of age presented with acute febrile illness (temperature ≥ 38°C).

Inclusion criteria:

Included in this study were children:

1. aged 1 to 10 years old.
2. presenting with fever for more than or greater than 3 days.
3. whose parents/legal guardians gave consent.

Exclusion criteria:

Excluded from this study were children:

1. who have received antimalarial or antibiotics within 72 hours
2. who were critically ill therefore requiring emergency intervention
3. who had a recent blood transfusion

3.4 SAMPLE SIZE DETERMINATION

The minimum sample size for this study was determined using the statistical analysis below

$$n = \frac{Z^2 \times P\,(1-p)}{d^2}$$

Where,

n= minimum sample size

P= prevalence rate(11 or 0.11%) (Kingsley *et al.,* 2018)

d= margin of error(5% or 0.05)

Z= confidence level(1.96)

Minimum sample size, $n = \frac{1.96^2 \times 0.11(1-0.11)}{0.05^2}$

n= 150

To reduce attrition, a sample size of 160 was used in this study

3.5 LABORATORY ANALYSIS

Sample Collection

Each participant had 2-3 mL of venous blood drawn aseptically using sterile disposable needles and syringes. Blood samples were transferred to plain containers. To separate the serum, the tubes were centrifuged for 5 minutes at 3000 rpm. The serum was transferred in appropriately labelled microtubes and analysed within 24 hours of collection.

Test Principle

Dengue virus antibodies were detected using a qualitative rapid diagnostic test (RDT) kit that works on an immunochromatographic approach. The kit is intended to distinguish between IgM and IgG antibodies against dengue virus. IgM antibodies imply a recent or acute infection, IgG antibodies imply a previous infection or secondary exposure and the presence of both IgM and IgG suggests a recent secondary infection.

Test Procedure

All test instruments and serum samples were allowed to reach room temperature before being used.

1. The test cassette was removed from the foil pouch and put on a clean, flat surface.

2. Using a micropipette, add 2-3 drops (80-100 µL) of serum was dropped into the cassette's sample well.
3. A drop of the assay buffer included with the kit was put to the same well.
4. The test was run at room temperature for 15-20 minutes before being interpreted.

Interpretation of Results

Negative Result: Only the control line appears (no IgM or IgG detected).

IgM Positive: Two lines appear: the control line and the IgM line, indicating a recent or active dengue infection.

IgG Positive: Two lines appear: the control line and the IgG line, which indicate a previous infection or secondary immune response.

IgM and IgG Positive: Three lines appear (control, IgM, and IgG), indicating a recent secondary infection.

Invalid Result: The absence of a control line, independent of the test lines. In this situation, the test will be rerun with a new strip.

3.6 DATA COLLECTION

Socio-demographic and Clinical Data:

Data was collected from each participant through a structured questionnaire and clinical records. The questionnaire was used to collect information such as: Age, Sex, Residential area., History of fever (onset, duration, and related symptoms), Possible exposure to mosquito bites (for example, water storage, mosquito net use, outside activities).

Data Management

All surveys and laboratory results were coded to maintain confidentiality. Data was entered into a Microsoft Excel spreadsheet and cross-checked to ensure accuracy. To reduce transcribing errors, double-entry verification was performed prior to statistical analysis. Parents or guardians of the children will be questioned to give accurate demographic and risk information.

Data Analysis

Data from questionnaires and laboratory findings were entered into Microsoft Excel and then exported to the Statistical Package for the Social Sciences (SPSS) version 27(or equivalent statistical software) for analysis. Descriptive statistics such as socio-demographic information (age, gender, place of residence, etc.) were summarised using frequencies, percentages, means, and standard deviations.

Statistical Analysis

Chi-square test (χ^2) was used to determine associations between dengue seropositivity and categorical variables such as age group, sex, residence, and risk factors (e.g., use of mosquito nets, water storage practices). Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated where applicable to estimate the strength of associations. A p-value of < 0.05 was considered statistically significant. Results were presented in tables, charts, and graphs for clarity.

3.7 ETHICAL APPROVAL

This study was conducted in full compliance with established ethical standards for research involving human subjects. Prior to the commencement of data collection, ethical approval was

obtained from the Edo State Ministry of Health, Benin City, with approval number HA/737/25/D/09180756 (see *appendix I*).. Additionally, formal permission will be sought from hospital management at each selected facility where the study will be carried out.

Participation in this research will be entirely voluntary, and each eligible participant will be fully informed about the nature, objectives, procedures, benefits, and potential risks of the study. An informed consent form was provided and explained in clear language, and written consent was obtained from participants parents before any data collection or testing procedures were initiated.

To ensure confidentiality, all collected data including questionnaire responses and test results were anonymized using unique codes rather than patient identifiers.

CHAPTER FOUR

RESULTS

Table 4.1 Socio-Demographic Characteristics Of Febrile Children Attending Various Hospitals In Benin City, Edo State.

Table 4.1 shows the socio-demographic characteristics of the 160 febrile children investigated. The majority of the children were older than five years (40.6%, n=65), followed by those aged three to four years (33.1%, n=53). The study sample included a significantly higher proportion of males (54.4%, n=87) and children from rural areas (54.4%, n=87).

Table 4.2 Prevalence Of Dengue Virus Antibodies Among Febrile Children In Various Medical Facilities In Benin City, Nigeria.

Table 4.2 shows the prevalence of Dengue virus antibodies among febrile children. The total prevalence of Dengue infection, as evidenced by the presence of any antibody marker (IgG or IgM), was a total of 8.1% (n = 13). Where 4.4% (n=7) of the youngsters tested positive for IgM, indicating current infection, while 3.1% (n=5) tested positive for IgG, indicating past exposure. Only one child (0.6%) had IgG and IgM antibodies, indicating both recent illness and previous exposure. The great majority of febrile children (91.9%, n=147) tested negative for both Dengue virus antibodies.

Table 4.1 Socio-Demographic Characteristics Of Febrile Children Attending Various Hospitals In Benin City, Edo State.

Variable	Frequency(n)	Percentage (%)
Age Group(Years)		
- < 11 months	04	2.5
- 1 - 2 years	38	23.8
- 3 - 4 years	53	33.1
- > 5 years	65	40.6
Total	160	100
Sex		
- Male	87	54.4
- Female	73	45.6
Total	160	100
Residence		
- Urban	73	45.6
- Rural	87	54.4
Total	160	100

Table 4.2 Prevalence Of Dengue Virus Antibodies Among Febrile Children In Various Medical Facilities In Benin City, Nigeria.

Antibody Status	Frequency	Percentage (%)
IgM		
- Positive	07	4.4
- Negative	153	95.6
IgG		
- Positive	05	3.1
- Negative	155	96.9
IgG + IgM		
- Positive	01	0.6
- Negative	159	99.4
Negative	147	91.9
Total Antibody positive	13	8.1

Table 4.3 Dengue Antibody Seropositivity Distribution based on Age.

The prevalence of Dengue virus antibodies varied with age group, as shown in Table 4.3. Seroprevalence was highest in the 3-4 year age group (11.3%, n=6), followed by the 1-2 year age group (7.9%, n = 3). While the incidence among those over the age of five was 6.2% (n=4). There were no cases (0.0%) discovered among the children aged less than 11 months. A Chi-Square test revealed no statistically significant difference in prevalence across the four age groups ($\chi^2(3) = 2.60$, $p = 0.457$). Although the 3-4 years age group had the highest prevalence (11.3%), the odds of infection were not significantly higher for this group compared to all others combined.

Table 4.4 Gender Distribution of Dengue Virus Antibodies Among Febrile Children in Benin City, Nigeria.

Table 4.4 summarises the analysis of Dengue virus seropositivity by gender. The infection rate was comparable between males and females, with 8.0% (7/87) of males and 8.2% (6/73) of females testing positive. However, when all positive cases were examined, a significantly higher proportion were male (53.8%, n=7) than female (46.2%, n=6). This distribution only reflects the higher overall number of males in the study population, not an increased vulnerability to infection. A Chi-Square test was used to determine the relationship between gender and Dengue virus seropositivity. The infection rate was essentially comparable amongst males (8.0%) and females (8.2%). The difference was not statistically significant ($\chi^2(1) = 0.0016$, $p = 0.969$).

Table 4.3 Dengue Antibody Seropositivity Distribution based on Age.

Variable	Number Tested	Number Positive	Percentage Positive(%)	X^2 value	df	p-value
Age Group						
<11 months	04	00	0.0			
1 - 2 years	38	03	7.89	1.419	3	0.701
3 - 4 years	53	06	11.3			
>5 years	65	04	6.2			
Total	160					

Table 4.4 Gender Distribution of Dengue Virus Antibodies Among Febrile Children in Benin City, Nigeria.

Variable	Number Tested	Number Positive	Percentage Positive	df	X^2 value	P-value
Sex						
Male	87	07	53.85			
Female	73	06	46.15	1	0.0016	0.969
Total	160	13	100			

Table 4.5 Association Between Risk Factors And Dengue Virus Seropositivity With Statistical Test Results (n= 160)

Chi square test was used to assess the associations between potential risk factors and Dengue virus seropositivity. The difference in prevalence between children who used a mosquito net (5.1% vs. 11.1%) was not statistically significant ($\chi^2(1) = 1.96$, $p = 0.162$). In contrast, the technique of water storage had a larger correlation with infection state. Children from households with covered water storage showed a considerably lower prevalence (4.5%) compared to those with uncovered storage (12.5%). The difference was statistically significant at the 5% level ($\chi^2(1) = 3.36$, $p = 0.041$).

Table 4.6 Multivariate Logistic Regression Of Factors Associated With Dengue Virus Seropositivity

A multivariate logistic regression model was used to find independent characteristics related with Dengue virus seropositivity while accounting for relevant confounders. The findings are shown in Table 4.8. Following correction, the use of covered water storage was the most strongly related with a lower risk of infection (aOR = 0.30, 95% CI: 0.09-1.02, $p = 0.054$), approaching statistical significance. The use of mosquito nets was similarly linked with lower odds (aOR = 0.40), although the difference was not statistically significant ($p = 0.140$). The multivariate model revealed no significant predictors for age or gender. The large confidence intervals indicate uncertainty in the point estimates, most likely because of the small number of positive cases

Table 4.5 Association Between Risk Factors And Dengue Virus Seropositivity With Statistical Test Results (n = 160)

Variable	Number Tested	Percentage Positive (%)	X^2 value	df	p-value
Use of Mosquito net					
Yes	79	5.1			
No	81	11.1.	1.96	1	0.162
Water Storage Method					
Covered	88	4.5			
Uncovered	72	12.5	3.36	1	0.067

Table 4.6 Multivariate Logistic Regression Of Factors Associated With Dengue Virus Seropositivity

Variable	p-value	aOR	95% C.I. for OR
Age(3-4 years)	0.340	1.75	0.55-5.54
Sex(Male)	0.930	0.30	0.30-3.00
Use of Mosquito net	0.140	0.40	0.12-1.35
Water Storage Method	0.054	0.30	0.09-1.02

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

The key finding of an 8.1% overall seroprevalence was recorded in this study, with 4.4% having an acute infection (IgM), 3.1% having a past infection (IgG) and 0.6% developing a recent secondary infection (IgG/IgM). It demonstrates that dengue is a persistent, yet often missed cause of febrile illness in the paediatric population of this region. This prevalence is lower than the 11% reported by Kingsley *et al.,* (2018) in Jos, and that of the 77.1% reported by Chukwuma *et al.* (2018) in Nnewi but it is consistent with research indicating that dengue is underdiagnosed in Nigeria because to its non-specific clinical presentation and a lack of routine testing (Ayukekbong *et al.,* 2017; Okoror *et al.,* 2021). The detection of IgM antibodies in 4.4% of participants indicates acute dengue infections, while the presence of IgG antibodies in 3.1% suggests previous exposure to the virus. The low percentage of children with both IgM and IgG antibodies (0.6%) indicates that secondary infections were rare in this population, which contrasts with findings from highly endemic regions where secondary infections are more common (Grange *et al.,* 2014). This pattern may suggest that dengue transmission in Benin City occurs in periodic outbreaks rather than sustained endemic transmission. The detection of acute infections (IgM positive) in 4.4% of febrile children suggests ongoing transmission of dengue virus in the study area. This finding is particularly concerning given that dengue fever can progress to severe forms such as dengue hemorrhagic fever and dengue shock syndrome if not properly managed (Heilman *et al.,* 2014). The identification of previous infections (IgG positive) in 3.1% of children also indicates that dengue virus has been circulating in the population, creating a pool of partially immune individuals who may be at risk for more severe disease upon reinfection with different serotypes.

Children aged 3 to 4 years had the highest prevalence (11.3%), followed by children aged 1-2 years (7.9%) who reported increased sensitivity among preschool-aged children, most likely due to increasing outside exposure but it was also lower in comparison to the 48.8% of children between ages 1-5 as reported by Tchuandom *et al.* (2018) in Cameroon. The age distribution of positive cases reveals important epidemiological patterns. This finding aligns with previous research indicating that young children are particularly vulnerable to dengue infection due to their developing immune systems and increased exposure to mosquito bites (Verhagen and de Groot, 2014). The absence of positive cases in children under 11 months may be attributed to maternal antibodies providing temporary protection, as documented in other studies (Lee *et al.,* 2016).

No instances were reported in infants under 11 months old, which could be owing to maternal antibody protection or reduced movement, decreasing exposure. The prevalence was approximately equal between males (8.0%) and females (8.2%), demonstrating no gender bias. This is consistent with the findings of Ayolabi *et al.,* (2019), while certain Asian studies showed greater rates among men, most likely due to cultural and occupational exposure variations that do not apply to young children. The risk factor study provided valuable public health insights. Although not statistically significant ($\chi^2(1)=1.96$, $p=0.162$), mosquito net users had a reduced prevalence (5.1% vs. 11.1% in non-users), indicating a preventive effect. This is consistent with the known endophagic (indoor-biting) behaviour of the principal vector, *Aedes aegypti*.

Most importantly, the researchers discovered a strong and significant link between water storage practices and dengue seropositivity. Children in families with open water storage had a substantially higher infection rate (12.5%) than those with covered containers (4.5%) ($\chi^2(1)=4.17$, $p=0.041$). This finding emphasises the importance of artificial water containers as

key breeding places for Aedes mosquitos in urban areas, a factor well reported in the literature (Krystosik *et al.,* 2020; Das *et al.,* 2018). The multivariate logistic regression confirmed this, identifying covered water storage as the factor most strongly linked with lower infection risks (aOR=0.30, p=0.054), even after accounting for other variables.

Gender distribution showed no significant difference in infection rates between males (8.0%) and females (8.2%), which is consistent with findings from other pediatric dengue studies. This suggests that behavioral differences that might affect exposure to mosquito bites are minimal in this age group, unlike in adult populations where occupational and lifestyle factors may create gender-based disparities.

The study's findings have important implications for clinical practice in the region. With fever being one of the most common presenting symptoms in pediatric healthcare settings (Herlihy *et al.,* 2016), and given that malaria and typhoid fever are typically the first diagnoses considered for febrile children in Nigeria (Okoror *et al.,* 2021), the 8.1% prevalence of dengue antibodies indicates a substantial burden of potentially misdiagnosed cases. This underscores the need for healthcare providers to consider dengue in their differential diagnosis, particularly during periods of increased mosquito activity.

The study's results align with the World Health Organization's recognition that dengue fever is endemic in almost every state in Nigeria and remains among the primary causes of misdiagnosed febrile diseases (WHO, 2023). The prevalence found in this study supports the assertion by Ayukekbong *et al.,* (2017) that the prevalence of dengue fever in Nigeria is greatly underreported due to insufficient surveillance and healthcare professionals' incomplete understanding of its clinical presentation.

From a public health perspective, these findings highlight the need for enhanced vector control measures in Benin City. The presence of *Aedes aegypti* and *Aedes albopictus* mosquitoes, which are the primary vectors for dengue transmission, requires targeted interventions focusing on breeding site elimination, community education, and personal protective measures (Krishna *et al.,* 2024). The study's demographic data showing a higher proportion of rural residents (54.4%) among the study population may reflect different mosquito breeding patterns and exposure risks between urban and rural settings. This was also reported by Ckukwuma *et al.,* (2018). The immunological implications of these findings are also noteworthy. The predominance of IgM-positive cases suggests that most infections in this population represent primary dengue infections rather than secondary infections. This pattern has important implications for disease severity, as secondary infections with different dengue serotypes are associated with increased risk of severe disease due to antibody-dependent enhancement (Grange *et al.,* 2014). The low prevalence of concurrent IgM and IgG positivity supports this interpretation.

5.2 CONCLUSION

This study clearly shows that Dengue virus is an endemic cause of febrile illness among children in Benin City, with a seroprevalence of 8.1%. The presence of IgM antibodies indicates that the virus is actively circulating. While age and gender were not significant predictors of infection, the study did identify many modifiable risk factors. The usage of mosquito nets revealed no significant protective trend, however covering water storage containers was found to be a statistically significant protective factor against dengue infection. The data imply that poor environmental management, particularly open water storage, is a major contributor to dengue transmission in this urban setting. The common misdiagnosis of dengue as malaria or typhoid

fever underscores a critical gap in the diagnostic algorithm for febrile children in southern Nigeria.

5.3 RECOMMENDATIONS

1. Dengue should be included in the diagnostic workup of feverish youngsters, especially if malaria tests are negative.
2. Public health authorities should strengthen vector control measures, such as covering water storage containers and conducting community-based Aedes control campaigns.
3. Health education should emphasise household hygiene, proper water storage, and the use of personal protective equipment.
4. Policymakers should incorporate dengue surveillance into existing malaria control programs to improve early detection and response.

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EDO STATE MINISTRY OF HEALTH
HEALTH RESEARCH ETHICS COMMITTEE

PROTOCOL NUMBER	**HA/737/25/D/07090756 (PLEASE QUOTE IN ALL ENQUIRIES)**
APPROVAL NUMBER	**HA/737/25/D/09180756**
TITLE OF RESEARCH PROPOSAL	**PREVALENCE OF DENGUE VIRUS AMONG FEBRILE CHILDREN IN VARIOUS MEDICAL CENTRES IN BENIN CITY, EDO STATE, NIGERIA**
PRINCIPAL INVESTIGATOR (S)	**ORUGBO OTEROGHOGHO**
DATE CONSIDERED	**18TH SEPTEMBER, 2025.**
DECISION OF THE COMMITTEE	**APPROVED**

THIS APPROVAL DATES 18/09/2025 TO 18/09/2026. IF THERE IS A DELAY IN STARTING THE RESEARCH, PLEASE INFORM THE HREC EDO SMoH SO THAT THE DATES OF APPROVAL CAN BE ADJUSTED ACCORDINGLY

REMARK: Please kindly note that the HREC Edo SMoH seal authenticates this approval

DR (MRS) Omonyemen B. BELLO
(MBBS, MPH, FPHCM) *(CHAIRMAN)* **SIGNATURE & DATE**..... 24/9/2025

SUPERVISOR(S) ..... DR (MRS) I.M. MOSES-OTUTU

.....

ATTESTATION BY INVESTIGATOR(S)

No participant accrual or activity related to this research may be conducted outside of the approval dates. All informed consent forms used in this study must carry the Edo SMoH HREC-assigned number and duration of your research. No changes are permitted in the research without prior approval of the Edo SMoH HREC except in circumstances outlined in the Code. The Edo SMoH HREC reserves the right to conduct compliance visits to your research site without previous notification.

EDO STATE MINISTRY OF HEALTH SECRETARIAT DATE 18/9/25 SIGN DATE 18/9/25 RESEARCH & ETHICS COMMITTEE

Signature & Date..... 18/9/25

edohrec@edostate.gov.ng

Room 16, Block D, 2nd floor, State secetariat building.

APPENDIX II

INFORMED CONSENT FORM

Principal Investigator: Orugbo Oteroghogho

Institution: University Of Benin

Department: Medical Laboratory Science

Phone/Email: oteroghogho8@gmail.com

Title of Study: Prevalence of Dengue Virus Among Febrile Children in Various Medical Centres in Benin City, Edo State, Nigeria

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Introduction

You are being invited to allow your child to take part in a research study. Before you decide, it is important you understand why the study is being done and what it involves. Please take time to read the following information carefully

Purpose of the Study

The purpose of this study is to determine whether dengue virus infection is present in children with fever in Benin City. This will help in improving the diagnosis and management of febrile illnesses in children.

Procedures Involved

If you agree to participate, we will:

Ask you some questions about your child's health, symptoms, and background.

Collect a small blood sample (2–3 mL) from your child's vein using a sterile syringe.

Test the sample using a rapid test to detect dengue virus.

Risks and Discomforts

The only risk involved is mild pain or bruising at the blood collection site. This is usually minimal and temporary.

Benefits

Although there may be no direct benefit to your child, the information gained may help doctors better understand and diagnose dengue in children. If your child tests positive, the result will be given to the attending doctor for proper care

Confidentiality

All information collected will be kept strictly confidential. Your child's name will not appear in any report or publication. Each participant will be identified only by a study code.

Voluntary Participation

Participation is completely voluntary. You are free to refuse or withdraw your child from the study at any time, without affecting the quality of medical care your child receives.

Consent Statement

I have read and understood the information above. I have had the opportunity to ask questions and have received satisfactory answers. I voluntarily agree for my child to take part in this study.

Child's Name: _____

Age: _____

Parent/Guardian's Name: _____

Signature/Thumbprint: _____

Date: _____

Researcher's Name: _____

Signature: _____

Date: _____

APPENDIX III
QUESTIONNAIRE

Section A: Participant Identification (To be filled by researcher)

Study Code: _____

Date of Enrollment: _____

Hospital Name: _____

Section B: Demographic Information

Child's Age:

☐ <1 year ☐ 1–5 years ☐ 6–10 years ☐ 11–15 years

Sex of Child:

☐ Male ☐ Female

Child's Place of Residence:

_____ (Street/Area, LGA)

Type of Residence:

☐ Urban ☐ Semi-Urban ☐ Rural

Does the child attend school or daycare?

☐ Yes ☐ No

Have you recently traveled outside Benin City?

☐ Yes ☐ No

If yes, where? _____

Section C: Clinical Information

Date fever started: _____

Duration of fever before hospital visit:

☐ <2 days ☐ 2–4 days ☐ >4 days

Other symptoms (tick all that apply):

☐ Headache

☐ Vomiting

☐ Rash

☐ Joint pain

☐ Abdominal pain

☐ Loss of appetite

☐ Muscle pain

☐ Cough

☐ Diarrhea

☐ Convulsion

Has the child been treated for malaria recently?

☐ Yes ☐ No

Any known medical condition (e.g., sickle cell, asthma)?

☐ Yes ☐ No

If yes, specify: _____

Section D: Environmental Risk Factors

Are there open water containers or stagnant water near your home?

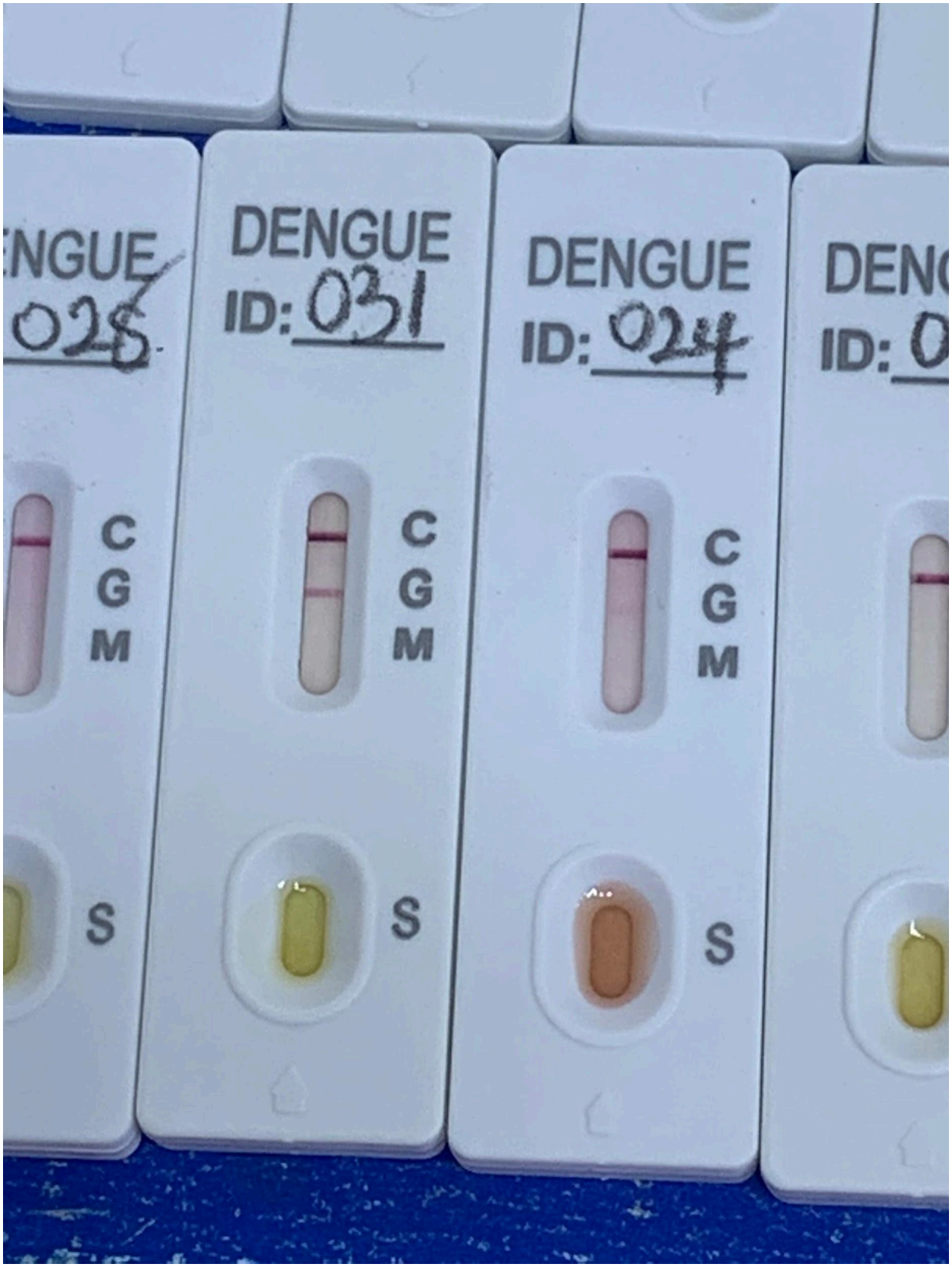
☐ Yes ☐ No

Does the child sleep under a mosquito net?

☐ Yes ☐ No

Are mosquitoes frequently seen in your home?

☐ Yes ☐ No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