

UNIVERSITY OF GHANA COLLEGE OF HEALTH SCIENCE



**TITLE OF THESIS: DENGUE INFECTION AMONG FEBRILE PEDIATRICS
POPULATION IN TWO SELECTED HOSPITALS IN BONO EAST REGION IN
GHANA.**

**THESIS BY: ADWOA TWUMWAAH GYAPON.
INDEX NUMBER: 11006627**

MPHIL: MEDICAL MICROBIOLOGY, UNVIVERSITY OF GHANA LEGON

DECLARATION

I, Adwoa Twumwaah Gyapon, authenticate that the work presented in this thesis is the result of my research undertaken at the Department of Medical Microbiology at the University of Ghana Medical School under the supervision of Rev. Prof Kwamena W.C. Sagoe and Dr. Mildred Adusei-Poku. I further declare that this thesis has neither in part nor whole been presented to the University or elsewhere for any degree, and that the authors whose works were referred to have been duly acknowledged.

Adwoa Twumwaah Gyapon

(Student)

Dr. Mildred Adusei-Poku

(Main Supervisor)

Rev. Prof. Kwamena W.C. Sagoe

(Co-Supervisor)

(Signature).

(Signature)

DEDICATION

I dedicate this work to my family, supervisors, friends and Amed (Japan Agency for Medical Research and Development) for their colossal contribution and support. I could not have accomplished this tremendous breakthrough without your unrelenting counsel and co



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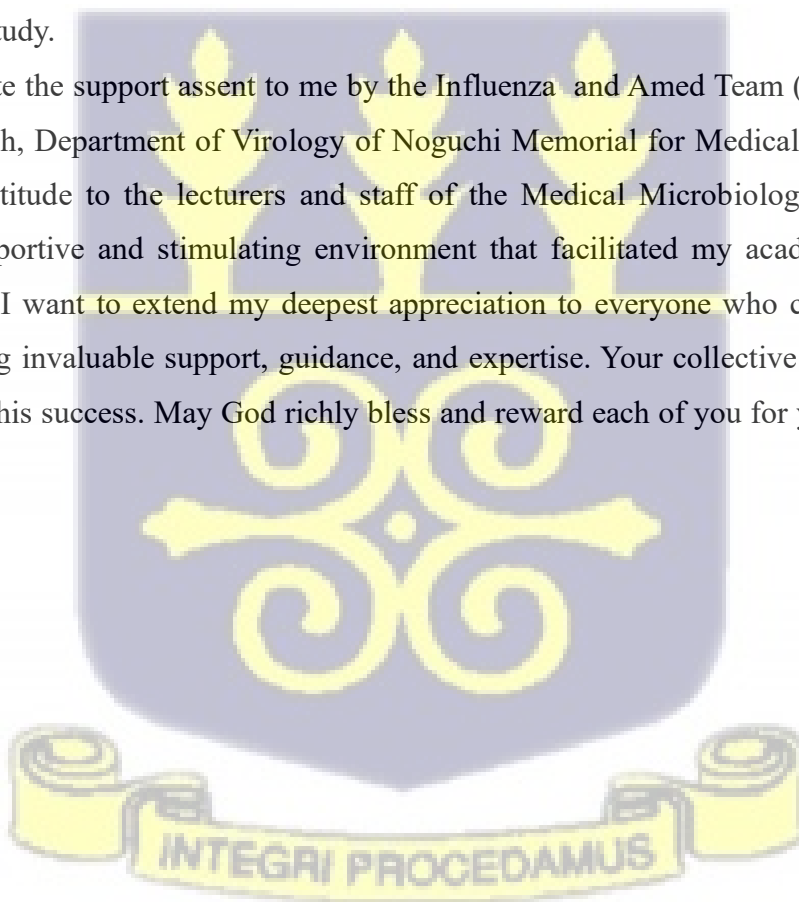
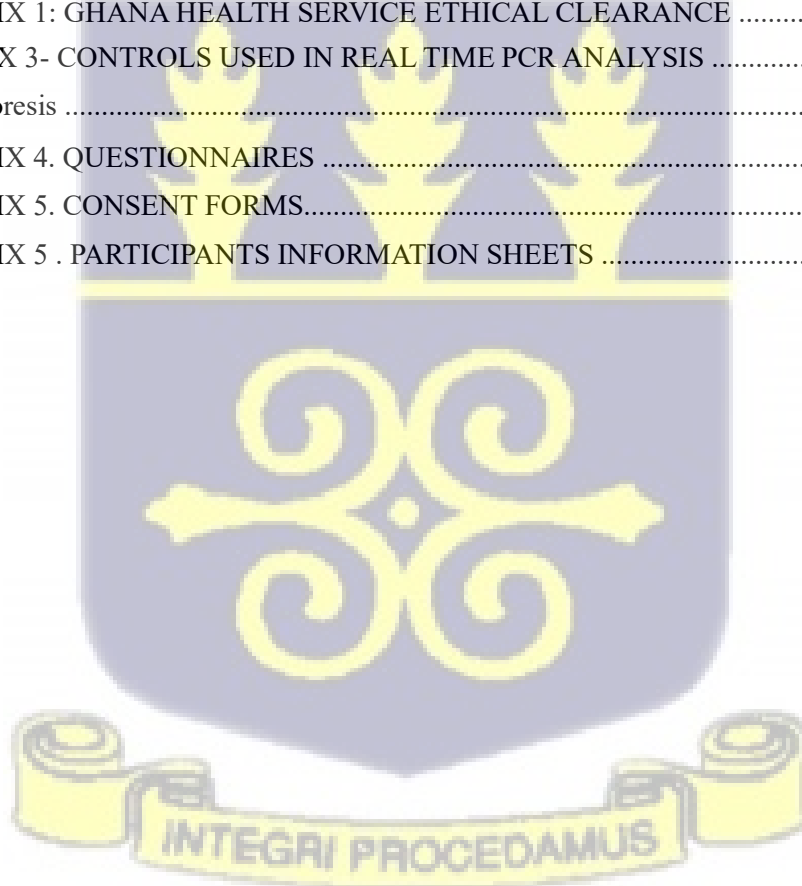


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ABBREVIATIONS AND ACRONYMS

DENV- Dengue Virus.

IgG-Immunoglobulin G

IgM-Immunoglobulin M

DF- Dengue Fever

DHF- Dengue Haemorrhagic Fever

DSS- Dengue Shock Syndrome

DENV-1- Dengue Serotype 1

DENV-2 -Dengue Serotype 2

DENV-3 -Dengue serotype 3

DENV-4 -Dengue serotype 4

RT-PCR- **Real- time Polymerase Chain Reaction.**

AFI- Acute Febrile Illness

WHO- World Health Organization.

RDT- Rapid Diagnostic Test.

ELISA- Enzyme linked Immunosorbent Assay.

TMB- tetramethylbenzidine.

DUS- Dengue Virus Universal Sense

DUC- Dengue Virus Universal Consensus.

OPD- **Outpatient Department**

CT- Cycle Threshold

NS1- Nonstructural 1

Ag- Antigen



ABSTRACT

Background

In Ghana, many febrile episodes are **routinely misdiagnosed** as malaria due to similar clinical presentations, insufficient knowledge, and inadequate diagnostic techniques. Dengue viral infection, primarily transmitted to humans by Aedes mosquitoes, represents a significant public health concern and remains the most widespread arboviral infection in tropical and subtropical regions (Bonney *et al.*, 2018). Dengue in febrile patients is characterised by symptoms such as myalgia, arthralgia, skin rashes, hemorrhagic tendencies, and, in severe cases, can lead to fatal outcomes.

The movement of individuals from neighbouring countries experiencing dengue outbreaks, combined with the widespread presence of Aedes mosquito vectors in Ghana, likely exacerbates the risk of dengue virus transmission. This shows the critical need for enhanced epidemiological surveillance and comprehensive risk assessment, particularly in high-risk areas like the Bono East Region. To address this emerging threat, it is imperative to improve diagnostic capabilities, increase awareness among healthcare providers, and strengthen public health responses to accurately identify and manage dengue cases.

Aim: **The aim of the study is** to determine dengue viral infection among **febrile pediatric patients** at the Atebubu hospital and Kintampo Hospital in Bono East region.

Results: The prevalence of dengue antigen, antibody and PCR were, 5.4%, 7.5% and 2.1% respectively. Males recorded high dengue antigen, antibody and PCR (5.93%), **(11.11%)** and (2.22%) respectively. Children aged group 1–3 years recorded the highest prevalence of dengue antigen (8.08%) while children aged group 4–5 years had the highest antibody (16.67%) and PCR prevalence (4.76%). Fever was the most common **symptoms** (13%) and water-collecting items

were marginally associated with dengue positivity. There was a correlation between dengue infection and **general fatigue in participants**. There was marginal correlation between dengue infection and participants with pains, cough, gastrointestinal disorder and refused meals. DENV1 was identified as the predominant serotype with CT values below 30 in most NS1-positive cases.

Conclusion: The findings emphasize the critical need for improved surveillance, diagnostic capabilities, and public health interventions to effectively combat dengue infections.



CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Dengue viral infection is a tropical disease transmitted by mosquitoes (Islam et al., 2021). This infection is prevalently observed in tropical and subtropical regions, especially in urban and peri urban environments. (Abdo Radman Al- et al., 2013.). The dengue virus belongs to the Flavivirus genus which is part of Flaviviridae family (Sinha et al., 2024). The dengue virus consists of four different serotypes: DENV-1, DENV-2, DENV-3, and DENV-4. (Bonney et al., 2018), (Murugesan & Manoharan, 2019). Importantly, Infection with one serotype does not confer immunity against the others (Beck et al., 2019). The dengue virus contains a positive-sense, single stranded RNA genome of approximately 10,700 nucleotides, encoding three structural proteins (capsid, membrane, and glycoprotein) along with seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) (Li & Kang, 2022). DENV genome **consists** of capsid, premembrane, and envelope by host and viral proteases (Li & Kang, 2022). Notably, NS1 plays a crucial role during the viral replication cycle, with a small portion remaining associated with intracellular organelles (Płaszczyc et al., 2019). Prior to the activation of an immune response during the initial infection, NS1 can be found simultaneously (Yusof, 2011). Infected cells release the viral protein NS1, which is highly concentrated in the infected individual's circulation. (Alcalá et al., 2018). Antibodies generated in response to NS1 viral infection have been shown to cross-react with different cell surface antigens (Chuang et al., 2016)

NS1 antigen including proteins found on endothelial cells and platelets (Chao et al., 2019).

This cross-reactivity has been implicated in the pathogenesis of dengue hemorrhagic fever (Jayatilaka et al., 2018). Dengue virus spreads to humans via the bite of *Aedes aegypti* and *Aedes albopictus* mosquitoes, which live in containers and tires (Banu et al., 2012). Moreover, rapid urbanization fosters more conducive environments for enhanced transmission, a rise in these vectors and possible adjustments in the ecological balance led to various strains (Duane J. Gubler et al., 2002). The viral illness in humans is a major public health problem with reported increased incidence worldwide in recent decades (Kofi Bonney et al., 2018)

Dengue fever results in an estimated 250,000 to 500,000 cases of dengue hemorrhagic fever each year, causing around 20,000 deaths annually, which corresponds to 264 disability-adjusted life years (DALYs) per million people (Van Dodewaard, 2015). Globally, 50–100 million dengue cases are reported each year in endemic regions (Manu et al., 2019a). Records show 390 million dengue infections per year with 96 million exhibiting clinical manifestations (Estofolete et al., 2019a). Approximately 3.9 billion individuals across 128 countries are vulnerable to dengue virus (Sigera et al., 2019). Estimates indicate that approximately 3.9 billion individuals are at risk of dengue viral infection across 128 countries (Alm et al., 2014).

In Africa, all four different serotypes of the dengue virus (DENV) have been identified; DENV2 is the most prevalent among them (Amarasinghe et al., 2011). There are 34 African countries where DENV transmission is known to be endemic (Were, 2012). Additionally, they have been identified in tourists from North America and Europe who are returning from several West African nations, including Ghana (Bulugahapitiya et al., 2007). The essential related factors for the cause of dengue infection are environmental, climatological and socioeconomic situations of a region (Sarker et al., 2024).

Dengue fever is prevalent across tropical regions, with varying levels of risk determined by environmental and anthropogenic factors including rainfall, temperature, relative humidity, and

rapid, unplanned urbanization (Bhatia et al., 2022). Before 1970, severe dengue epidemics were limited to only nine countries, according to the World Health Organization (Murray et al., 2013a). Nevertheless, the disease is endemic in more **than** 100 countries spanning several WHO regions, including Africa, the Americas, the Eastern Mediterranean, South-East Asia and the Western Pacific. (Guzman et al., 2016). The regions most impacted by the disease include the Americas, **Southeast** Asia, and the Western Pacific, with Asia contributing to approximately 70% of the global disease burden. (Zerfu et al., 2023).

Dengue virus often spreads via **travellers** who introduce the virus to areas with competent mosquito vectors, increasing the potential for local transmission. (Wilder-Smith, 2012). The heightened movement of infected individuals across continents has led to an increased number of cases emerging in previously unaffected areas, including Europe, where explosive outbreaks are now a concern, potentially leading to dengue outbreaks in multiple European countries (Lessa et al., 2025). Dengue fever outbreaks **are** prevalent across various nations **in** the Americas, the Eastern Mediterranean, South-East Asia, and the Western Pacific regions (Guo et al., 2017a).

However, Africa has not reported significant outbreaks, with only **a few countries** reporting cases (Amarasinghe et al., 2011b). Moreover, the rapid spread of DENV fever in Africa, especially in East Africa, is alarming, with severe epidemics impacting urban populations (Agha et al., 2017). Recent outbreaks in Somalia (2011, 2013) resulted in co-**circulation** of multiple dengue serotypes which may lead to death (Agha et al., 2017). **Several** factors influence the ongoing transmission of dengue virus (DENV) in Africa, including the efficiency of mosquito vectors, urbanization, and the presence of nonbiodegradable containers that create breeding sites. Additionally, the virus's infectivity and host susceptibility are important in this dynamic (Amarasinghe et al., 2011c). However, dengue typically presents in its early stage, the illness presents with a mild, influenzalike

fever, often mimicking other infectious diseases such as influenza, measles, Zika virus, chikungunya, yellow fever, and malaria (Farias et al., 2024).

This similarity can make diagnosis challenging. (Muller et al., 2017a). The dengue virus has an incubation period of approximately 4 to 7 days. Primary infection with any of the four serotypes (DENV1–DENV4) typically results in dengue fever (DF), an acute, self-limiting viral illness characterized by high fever and muscle pain, though it can also present asymptotically. (Dorman et al., 2004). However, the intensity of a dengue infection might range from a minor sickness (febrile fever) to dengue shock syndrome (Chaloemwong et al., 2018). Infection with different serotypes of the virus can result in dengue hemorrhagic fever (DHF), a severe condition characterized by plasma leakage, low platelet counts, and occasionally hypovolemic shock (Simmons et al., 2005).

Dengue shock syndrome (DSS) can cause issues with coagulation, plasma leakage, and heightened vascular fragility in the human body (Bhatt et al., 2021a). Increased in capillary permeability due to fluid loss might result in **multi-organ** failure which will lead to death (Pang et al., 2017). Following the initial dengue infection, IgM antibody levels typically increase significantly within 3 to 5 days after the onset of fever. These antibodies typically last for 30 to 60 days (Sang et al., 1998). Conversely, in cases of subsequent dengue infection, DENV IgG antibodies rapidly develop and reach elevated levels shortly after the fever begins. (Murugesan & Manoharan, 2019b).



1.2 Problem statement

Dengue viral infection poses a significant and growing public health challenge in Ghana, particularly among febrile patients in hospitals within the Bono East Region. **Despite the increasing incidence of dengue, many cases go underreported or undiagnosed due to its clinical similarity with other common febrile illnesses, such as malaria and typhoid fever (Chipwaza et al., 2014a).** This overlap complicates accurate diagnosis and treatment, as febrile patients are often misdiagnosed due to the limited availability of proper diagnostic tools and a general lack of awareness among healthcare providers.

From January 2022 to December 2023, dengue cases were reported across all 16 regions of Ghana, with evidence of DENV-1 circulation during this period (Bonney et al., 2024). Previous years had also shown dengue circulation, with DENV-2 and DENV-3 commonly detected. **A serological study in the Central Region revealed a high prevalence of anti-dengue IgG and IgM antibodies among hospital attendees (Kofi Bonney et al., 2018a; Amoako et al., 2018b), further indicating ongoing transmission (Aniakwaa-Bonsu et al., 2021a)**

Similarly, in the Bono East Region, particularly at Kintampo Municipal Hospital, dengue seroprevalence studies have shown that 3.2% of children had current exposure, while 21.6% have been previously been exposed to the dengue virus (Stoler et al., 2015). Despite this evidence of dengue presence and circulation in the Bono East Region, there remains a significant gap in understanding its true burden among febrile patients. Although prior exposure to the virus has been demonstrated through seroprevalence studies, active surveillance and detection efforts remain inadequate. The situation is further complicated by the presence of other arboviral diseases, such as yellow fever, which are also transmitted by the same *Aedes* mosquitoes (Agboli et al., 2022).

These factors underscore the urgent need for comprehensive studies to assess dengue's prevalence, diagnostic challenges, and impact among febrile patients in selected hospitals within Bono East. Addressing these challenges is crucial for formulating effective public health strategies to control and manage dengue in the region.

1.3 Justification.

The rising frequency of dengue in the Bono East Region of Ghana highlights the critical need for research to assess the prevalence of dengue among febrile patients in local hospitals. With dengue cases on the rise across Ghana, including in Bono East, it is essential for health authorities to prioritize this issue. Comprehensive data from this study will inform diagnostic practices and guide public health interventions to effectively manage and reduce the burden of dengue in the region.

1.4 Aim of the Study

The aim of the study is to determine dengue viral infection among febrile pediatric population in two selected hospitals in Bono East region.

Specific Objectives of the Study

- To determine the prevalence of dengue antibodies and antigen among febrile pediatrics in the two selected hospitals in Bono East of Ghana.
- To determine the predictors for dengue viral infection among febrile pediatrics in the two selected hospitals in Bono East of Ghana.



CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Dengue virus

Dengue viruses are part of the Flavivirus genus within the Flaviviridae family (Sinha et al., 2024). This genus includes other viruses such as yellow fever, West Nile, and Japanese encephalitis. (Amarasinghe et al., 2011b). These viruses are transmitted by mosquitoes and cause diseases (Carrington & Simmons, 2014a). The dengue virus was first identified by Ren Kimura and Susumu Hotta in 1943 from blood samples collected during a dengue epidemic in Nagasaki, Japan. (Rahmat, 2022).

2.2 The Dengue Serotypes

There are four closely related dengue virus serotypes that cause dengue illnesses, namely Dengue 1 to 4 (DENV 1- 4) (Bhatt et al., 2021b). These four serotypes interact differently with antibodies in human blood serum (Dey & Mukhopadhyay, 2017). Although the genomes of the dengue viruses are similar—sharing approximately 65% of their genetic material—there is still notable genetic diversity within each serotype (Dieng et al., 2021). Contracting a particular serotype triggers a durable antibody-based immune reaction specific to that serotype; however, this immunity does not extend to other serotypes (Yong et al., 2022). Despite these variations among serotypes, dengue infection consistently leads to the same illness and a similar range of clinical symptoms across all types (Gubler et al., 1998a). All four serotypes of dengue were recognized in Southeast Asia in the 1970s, while DENV1 and **DENV2** were discovered in Africa and Central America (Cucunawangsih &

Lugito, 2017). Currently, all four dengue serotypes coexist in regions of tropical and subtropical climate (Cucunawangsih & Lugito, 2017). The four serotypes of dengue virus share similar ecological and geographical environments (E. Khan et al., 2020).

2.3 Dengue fever and its transmission.

Dengue fever is primarily transmitted to humans via the bite of an infected *Aedes* mosquito (Mat Jusoh & Shueb, 2017): (Malavige et al., 2004). The *Aedes aegypti* mosquito feeds on an infected person during the viremic phase of the illness, and the virus is spread. (Carrington & Simmons, 2014b)

. Symptoms typically appear two days before the fever begins and can last for four to five days after

the fever starts. (Long et al., 2019). After the mosquito ingests the virus infected blood, it attaches to specific receptors on the midgut epithelial cells (Carrington & Simmons, 2014b). The virus replicates within the midgut and is subsequently released into the hemocoel, the virus disseminates to various secondary tissues, including the salivary glands (Carrington & Simmons, 2014b). When the salivary glands become infected, the virus can be transmitted to a new host through the saliva of the infected mosquito during its next blood meal (Carrington & Simmons, 2014b). After the virus incubation period, the infection progresses through three phases: the febrile phase, the critical phase, and the recovery phase (Rajapakse et al., 2012). The condition is marked by a fever that typically lasts between 2 and 7 days, followed by a range of non-specific symptoms (Gulati & Maheshwari, 2012). These may include headaches, pain behind the eyes, vomiting, loss of appetite, muscle aches, nausea, and discomfort in the joints (Khosavanna et al., 2021). The variety of symptoms can make it challenging to distinguish this illness from other febrile conditions (Kalayanaroop et al., 1997). And towards the end of the febrile phase, the rash often

transitions to petechiae, which can manifest on the patient's feet and hands. (Estofolete et al., 2019b). The critical phase is characterised by rash on the body that may be maculopapular (S et al., 2021).

The critical phase lasts from 2 to 3 days since the onset of the fever (Kularatne, 2015). In this stage, there is plasma leakage with or without hemorrhage. There is also an increment of capillary permeability and **rise** of hematocrit level (Estofolete et al., 2019b)

The infection can progress to several conditions, including undifferentiated febrile illness, Dengue fever, Dengue hemorrhagic fever, and Dengue shock syndrome (Gurugama et al., 2010). Undifferentiated febrile illness cannot be diagnosed solely based on clinical observations and requires serological or virological diagnostic methods for accurate identification. (Zerfu et al., 2023b). Dengue fever (DF) is characterized by several key symptoms, including a high fever that can reach 40°C (104°F), intense headaches, and retro-orbital pain, as well as in the muscles and joints (Khosavanna et al., 2021). Additional symptoms may include nausea, vomiting, swollen glands, and the occurrence of a rash (Ahmed et al., 2001; Kittigul et al., 2007). The severity of the disease is influenced by patient's age, race, and secondary infection by a different serotype (Dhanoa et al., 2016). Dengue fever is common in adults and children (Gurugama et al., 2010a).

Dengue Hemorrhagic Fever (DHF) and Dengue Fever (DF) are distinguished mainly by an increase in vascular permeability, which results in plasma leakage (Srikiatkachorn, 2009). However, symptoms during the febrile phase resemble those of Dengue Fever (Kalayanaroj et al., 1997). Dengue Shock Syndrome (DSS) represents the most critical manifestation of dengue infection with a mortality rate of up to 20% if not properly treated (Owais et al., 2023). In DSS patients, leakage ranges from mild, manageable with initial resuscitation, to severe, requiring intensive care and extensive fluid therapy for complications (Lam et al., 2015).

The recovery phase is marked by the reabsorption of fluid and typically lasts for 2-3 days (Tejo et al., 2024). Infection with any of the four dengue serotypes can induce a variety of ailments after a 4-to-7-day incubation period (Bhatt et al., 2021c).

2.4 Epidemiology of Dengue and Regional Trends of Dengue Infection

2.4.1 Global burden of Dengue Infection

Dengue has emerged as one of the most significant mosquito-borne viral infections worldwide. Reported cases increased from about 505,430 in 2000 to 5.2 million in 2019 (Yek et al., 2023). Nevertheless, this represents only a fraction of the true burden, as many as most infections are mild or asymptomatic or misclassified as other febrile illness (Pawar, 2015; Ullah et al., 2023). Moreover, over the past fifty years, dengue viral infections have increased thirty-fold, potentially affecting as many as 3.97 billion people each year (Samal et al., 2020). Several factors particularly rapid urbanization, globalization, and climate change have further contributed to this rising trend, thereby leading to an increasing tendency of dengue viral cases (Gubler et al., 2011). In addition, recurrent and serious epidemics of dengue expands to new regions, including Europe (Akinsulie & Idris, 2024). In 2010, imported cases were identified in three European countries, and local transmission was first recorded in France and Croatia (Grandadam et al., 2011; Emmanouil et al., 2020). Overall dengue remains endemic in WHO regions with recurrent epidemics posing substantial public health and economic burdens (Fredericks & Fernandez-Sesma, 2014).

2.4.2 Regional Trends of Dengue Infection

2.4.2.1 The America

Several US-associated territories where dengue is endemic including Puerto Rico, the Virgin Islands, American Samoa, and other US-affiliated Pacific Islands have frequent co-circulation of various Dengue virus serotypes (Santiago et al., 2013). Furthermore, between 1970 and 2007, dengue fever cases in the Americas rose to over 900,000, while reported cases of dengue hemorrhagic fever exceeded 25,000 (Dengue Reborn Widespread Resurgence of a Resilient Vector Environews | Focus,2008.). In addition, Cuba experienced a notable Dengue epidemic in 1981 where 344,203 cases were documented and reported daily to the Ministry of Public Health (Dick et al., 2012; Kouri et al., 1986.). Likewise, Brazil, Colombia, and Venezuela have also documented major outbreaks since 2000 (Wilson & Chen, 2002). Consequently, dengue is now hyperendemic in America, with all four viral serotypes present (Murray et al., 2013b). However, a persistent challenge in the region is the cyclical nature of outbreaks despite extensive vector control programs.

2.4.2.2 Asia and Western Pacific

Asia bears the highest burden of dengue accounting for the majority of the global cases. Moreover, studies across Southeast Asia highlight recurrent epidemics every 2-5 years with hyperendemic circulation of multiple serotypes (Prasith et al., 2013). For Instance, Indonesia has the highest incidence, whereas Singapore has observed a shift with over half of fatalities occurring in individuals aged 15 and above since 1982 (GuhaSapir & Schimmer, 2005). In addition, children remain disproportionately affected with hospitalbased surveillance in Philippines and Vietnam reporting dengue as leading cause of pediatric morbidity and mortality (Alejandria, 2014). Conversely, during Bangladesh's 2000 outbreak,82% of cases occurred in adults while a smaller proportion involved individuals under 18 years old. (Hossain et al., 2023). Furthermore, Other countries-

dengue, such as Nepal, Bhutan, Macau, Hong Kong, Taiwan, Madagascar, the Galápagos Islands, and Easter Island, have experienced outbreaks of the disease (Chowdhury & Riddell, 2015)

Gender-related disparities likewise have also been observed, with higher infection rates among males, possibly due to greater outdoor exposure linked to gender roles (Anker & Arima, 2011). Taking together these patterns, this indicates changing immunity profiles in populations, yet regions-wide studies that integrate demographic shifts with viral evolution remain limited.

2.4.2.3 Africa

Dengue has been long present in Africa for decades, though its true burden remains underestimated. No estimates are currently available for dengue virus infection rates in Africa (Franco et al., 2010). Dengue fever has been reported in 34 countries, all of which have populations of *Aedes* mosquitoes in Africa (Amarasinghe et al., 2011). However, the four distinct serotypes have been detected in Africa (Amarasinghe et al., 2011). In the year 1960 and 2010, 2020 outbreaks of dengue were recorded in fifteen African countries and the majority happening in Eastern Africa (Amarasinghe et al., 2011b). In addition to previous report, Dares Salaam in Tanzania had become a major focus for dengue activity with outbreaks and large epidemics in 2014 involving 1018 confirmed cases (Mboera et al., 2016). Importantly, DENV-2 was identified as the causative agent of the 2014 outbreak in Tanzania, however, cases of DENV-3 being exported from Tanzania were also documented further underscoring the country's role in regional and international transmission of dengue (Okada et al., 2019). In Kenya, there is growing evidence supporting the presence of dengue, as local data indicate its identification as the most prevalent viral pathogen in blood samples retrospectively tested from HIV-negative

survey participants (Lim et al., 2020). Moreover, these findings highlight the increasing recognition of dengue as a significant public health concern in the region.

2.4.2.4 Epidemiology of Dengue in West Africa.

In west Africa, the epidemiological data on dengue is limited with regional distribution and transmission threats projected via ecological models (Messina et al., 2019). During yellow fever outbreak in Cote d'Ivoire in 2008, the co-circulation of dengue was observed, with 34 individuals testing positive for IgM antibodies: nine showed dual positivity for both yellow fever and DEN IgM, three were specific for yellow fever, and 22 were positive for DEN IgM (Akoua-Koffi et al., 2014). Building on this, a prospective study in Cote D'Ivoire sought to estimate the proportion of dengue and malaria among febrile patients. The findings revealed that three patients (0.4%) had laboratory confirmed dengue, with two testing positive for anti-dengue IgM and one confirmed by RT-PCR (L'Azou et al., 2015). In Ouagadougou, Burkina Faso, the presence of three dengue serotypes (DENV-2, DENV-3 and DENV-4) was detected among febrile patients who tested negative for malaria by rapid diagnostic tests. This finding highlights the circulation of multiple dengue serotypes in the region, underscoring the need for improved arboviral surveillance where malaria is often the default diagnosis (Ridde et al., 2016).

Several studies have documented the circulation of dengue virus in different regions of Nigeria, highlighting its public health importance despite being often misdiagnosed as malaria. A study conducted in Ogbomoso reported a high seroprevalence of anti-dengue antibodies (28.6%) among individuals aged 0-15 years with higher rates among males

(18.9%), civil servants (26.3%) and urban inhabitants (21.3%). Febrile patients between 21-40 years also showed significant infection rates (Oladipo et al., 2014). Further supporting these findings, in Ilorin, a 2016 study reported a striking 46.0% seroprevalence of anti-DENV IgM antibodies, with 2.84% of cases involving concurrent malaria infection. Additionally, 48 of 95 IgM-negative samples were positive for DEN IgG, and 11 (6.25%) samples were confirmed DEV-positive by RT-qPCR (Kolawole et al., 2017). A study was conducted to identify dengue serotypes and genotypes among febrile patients in two health facilities in Lagos: however only eleven individuals (8.5%) tested positive for dengue virus (Ayolabi et al., 2019). Importantly, dengue virus serotypes 1 and 3 were found to be circulating in Lagos, Nigeria. Similarly, a serological survey of nineteen DENV-2 were isolated from children, with prevalence increasing steadily with age (Onoja et al., 2023). Consistently, a study in Oyo state reported both IgM and IgG antibodies, suggesting past exposure and recent circulation, with co-detection of LASV and DENV highlighting risks of co-infection (Animasaun et al., 2025). Similar patterns have been observed elsewhere in West Africa. In Cameroon, dengue virus was detected in 114 febrile patients at the New-Bell District Hospital in Douala, with eight cases of DENV only infection and 13 cases of dengue-malaria co-infection, indicating overlapping transmission of both pathogens (Yousseu et al., 2018). Summarily, in 2018, 14.3% of residents in the Duodji health area tested positive for dengue serotype 1, a trend attributed to the abundance of Aedes Mosquitoes, favorable climatic conditions and socio-economic factors facilitating transmission (Nemg Simo et al., 2019). Following this a pilot study in Senegal reported high prevalence of viral pathogens among children with non-malarial fever, with dengue virus isolated from 55 cases (Boris et al., 2021). This underscores the expanding burden of dengue in the region and the need for

integrated surveillance beyond malaria. Despite all the outbreaks recorded, the data on prevalence and incidence are under reported and underrecognized since they mostly misdiagnosed as malaria (Were, 2012)

2.4.1.2 Epidemiology of Dengue in Ghana

Ghana is located on Africa's west coast, between latitudes 4° 44' N and 11° 15' N, and longitudes 1° 12' E and 3° 15' W. It spans an area of approximately 239,460 km² and is bordered by Côte d'Ivoire to the west, Togo to the east, Burkina Faso to the north, and the Atlantic Ocean (Gulf of Guinea) to the south (<https://hdl.handle.net/10568/40030>) . The country is divided into ten administrative regions and characterized by diverse landscapes, including grasslands, shrublands, forests, plains, rivers, waterfalls, and offshore islands (Appiah et al., 2014). Ghana experiences a tropical climate with regional variations: the eastern coast is warm and arid, the southwest hot and humid, and dry, with wet and dry seasons defining rainfall distribution (Edmund I. Yambah, 2023.) Dengue virus circulation in Ghana has been documented over the past two decades. In 2005, DENV-2 was detected in a traveler returning from Ghana (Huhtamo et al., 2008.) Subsequently, dengue-specific IgM was identified in 3.2% and IgG in 21.6% of malaria-positive children in Navrongo, Kintampo and Accra (Stoler et al., 2015). In addition, acute DENV-2 infections were later confirmed in Accra, genetically similar to strains from Burkina Faso, indicating local transmission (Amoako et al., 2018). Furthermore, both DENV-2 and DENV-3 were detected among patients suspected of Ebola virus disease, with DENV-3 closely related to strains from Senegal and India (Kofi Bonney et al., 2018) . Similarly, arboviral circulation was confirmed in Accra, where 69.23% of febrile patients tested positive for dengue-specific antibodies (Manu et al., 2019).

More recently, seroprevalence studies revealed DENV-IgM and IgG in children, with rates of 12.6% and 2.2% respectively (Aniakwaa-Bonsu et al., 2021). Despite the evidence of dengue circulation in various parts of Ghana, little is known about its presence in Bono East Region. Moreover, Bono East, one of the recently created region, is situated in the middle belt of Ghana and characterized by tropical climatic conditions with distinct wet and dry seasons, favorable for mosquito breeding. In addition, the region is large agrarian, with irrigation schemes, stagnant water bodies, and peri-urban settlements that provide suitable habitats for *Aedes* mosquitoes, the primary vectors of dengue virus. For instance, a study was conducted in Accra which reported dengue-Specific IgM (3.2%) and IgG (21.6%) of malariapositive children in Navrongo, Kintampo and Accra (Stoler et al., 2015). However, no comprehensive study has been conducted in Bono East to determine the burden of dengue virus infection, particularly among children presenting with febrile illness. Consequently, given that malaria is hyperendemic in the region, febrile cases are often presumed to be malaria-related increasing the risk of misdiagnosis and underreporting of dengue and other arboviral infections. This knowledge gap underscores the importance of establishing baseline data on dengue circulation in the Bono East region.

2.4.1.3 *Aedes* Mosquito Vector for Dengue Virus Transmission in Ghana.

A study reported that more than 56% of homes surveyed in Ghana's northern belt tested positive for *Aedes* larvae (Appawu et al., 2006). In a separate study was conducted to map *Aedes* mosquito vectors in the Ashanti region, located in Ghana's middle belt, discovered that *Aedes aegypti* was the dominant species, accounting for 81% of the population (Esen et al., 2013.).

Similarly, a study found a large population of *Aedes aegypti* in a port city in Accra with high frequencies of insecticide resistance mutation (C. Ojukwu et al., 2022). Moreover,

another investigation aimed at assess the potential risk of yellow fever, dengue, chikungunya, and Zika virus transmission, conducted in the Larabanga and Mole Game Reserve areas of Northern Ghana, 99.22% of the collected *Aedes* mosquitoes were identified as *Aedes aegypti* (Joannides et al., 2021). However, upon examination using RT-PCR, these mosquitoes tested negative for dengue, Zika, and chikungunya viruses (Joannides et al., 2021). Similarly, a crosssectional study was conducted to evaluate the risk of dengue, Zika, and chikungunya transmission within domestic and peri domestic populations in the Greater Accra Region revealed a high prevalence of *Aedes aegypti* (Akyea-Bobi et al., 2022).

2.5 The effect of Climate change, Urbanization and Population Dynamics on dengue fever in Ghana

Mosquito population dispersal, community makeup, interactions with hosts, and infection rates are all driven by urban environment (Kolimenakis et al., 2021). *Aedes* mosquitoes are important vectors for public health concerns because of their vector competence, close relationship with human populations, and ability to feed on human blood (Khezzani et al., 2023). Hence, to **minimizing** the risk of arbovirus transmission to humans requires a better understanding of how urban environments affect mosquito abundance, feeding habits, and arbovirus infection status (Little et al., 2022).

Climate change and globalization exert direct influence on the dissemination of mosquitoes and are widely acknowledged as significant determinants affecting the transmission of arboviruses (Obame-Nkoghe et al., 2024). *Aedes aegypti*, for instance, stands out as a primary mosquito vector responsible for the global distribution of arboviruses, with its origin traced back to Africa (Gómez et al., 2022). Conversely, *Aedes albopictus*, identified as a mosquito vector in various regions, was introduced to Africa approximately three decades ago (Amoa-Bosompem et al., 2021).

Fluctuations in Aedes population density are anticipated seasonally, with decreased numbers during dry periods, escalating with elevated temperatures, and the potential for more abundant breeding sites during the rainy season (Owusu-Asenso et al., 2022).

2.6 Clinical presentation of dengue fever

Many patients experience an initial phase characterized by chills, rash, and maybe other associated symptoms such as facial flushing, being a distinctive and reliable indicator of dengue fever, often lasting 3-7 days (Gurugama et al., 2010b)

Classical dengue fever represents an acute infection that emerges within a period of 4 to 10 days **after** the bite of an infected mosquito (Muller et al., 2017b).

Children aged 15 and younger often have a nonspecific febrile illness characterized by a rash of tiny, raised patches (Barbi et al., 2017). Dengue fever typically begins suddenly and is characterized by fever, chills, and severe discomfort in the head, back, and limbs, also known as "breakbone" agony, among other symptoms. **A fever that lasts more than ten days is unlikely to be caused by dengue (Gubler et al., 1998c)** The symptoms of dengue fever present with varying severity, from a mild febrile illness to severe manifestations such as dengue hemorrhagic fever (DHF) and **dengue shock** syndrome (DSS). (Zhang et al., 2014). The patient obtains immune protection against the disease caused by the particular dengue virus serotype after the initial infection (Halstead SB, 1974). Individuals infected with a different serotype of the dengue virus are at a higher risk of developing severe dengue symptoms (Henchal & Putnak, 1990). Symptoms in individuals may include pain, fever, headache, eye pain, body aches, nausea, vomiting, skin rashes, fatigue, loss of appetite, sore throat, swollen lymph nodes, and mild bleeding (Heilman et al., 2014).

Fever usually decreases as the viremia fades. In certain cases, especially in youngsters, fever may subside for a day and then return, a pattern known as saddleback fever (Gubler et al., 1998d)

Hemorrhagic manifestations during the acute febrile phase can **range from a positive** tourniquet test and the presence of petechiae to the occurrence of spontaneous gastrointestinal bleeding in nasal, oral, and other mucosal areas (Antunes et al., 2013). The severity of symptoms at this stage does not always signal a progression to severe dengue (Muller et al., 2017b). As a result, diligent monitoring of early warning symptoms is essential during the critical phase of the illness (<https://www.cdc.gov/dengue/hcp/clinical-signs/index.html>).

Most DENV-infected patients recover completely after the febrile stage and do not progress to the critical phase of the disease (Muller et al., 2017b). Patients entering the critical phase may display early signs of increased capillary permeability, potentially resulting in plasma leakage (Weiss et al., 2006). Patients' condition often worsens during the time of defervescence, which occurs around day 4 of illness, when their temperature drops to between 37.5°C and 38°C, and this is when the first signs of vascular leakage appear may manifest (Muller et al., 2017b).

2.6.1. Fevers of unknown origin and Malaria burden among febrile patients.

Malaria misdiagnosis is a persistent issue in sub-Saharan Africa, particularly affecting impoverished communities (Stoler & Awandare, 2016a). In high-transmission, resource-limited settings, healthcare facilities have traditionally relied on clinical algorithms for malaria diagnosis and management (Tarimo, 2017). A clinical algorithm for malaria diagnosis is based on a defined set of symptoms and signs, such as cyclical fever, chills, and hepatosplenomegaly (Stoler & Awandare, 2016b). Due to the similarity in symptoms between non-severe malaria and other acute febrile illnesses, clinical algorithms often lead to false positives and missed cases, hindering accurate differential diagnosis (Ye et al., 2009).

In sub-Saharan Africa, diseases such as typhoid, influenza, hepatitis, and dengue are likely underreported, as fever is frequently misattributed to malaria and treated as such, even in the absence of malaria transmission (Ye et al., 2009). However, ensuring healthcare provider adherence to the 2010 WHO guidelines can be challenging (Stoler & Awandare, 2016b). A Tanzanian study on acute febrile illness (AFI) found that of 870 hospital admissions, 60.7% were clinically diagnosed with malaria. However, only 1.6% had confirmed malaria parasitemia, while other infections (bacterial, fungal, and arboviral) were misdiagnosed as malaria due to similar clinical presentations (Boillat-Blanco et al., 2018). Overdiagnosis of malaria in sub-Saharan Africa is influenced by high clinical workloads, inconsistent healthcare worker training, and variable laboratory techniques, alongside the use of both modern and traditional medicine. As a result, clinical data significantly underrepresents the true burden of acute febrile illness (AFI) in the region (Wongsrichanalai et al., 2007).

In hyper- and holoendemic regions, naturally acquired immunity helps protect adults from severe malaria, allowing them to maintain a low-level parasite burden that would be fatal to malaria-naïve individuals, without suffering significant disease (Doolan et al., 2009). This complicates the diagnosis of acute febrile illness, as patients presumed to have malaria may test positive on a sensitive malaria rapid diagnostic test due to a low parasite load, despite having a different infection. (Stoler & Awandare, 2016b).

In Kezdoglou, south-eastern Senegal, a study was conducted to investigate the concurrent malaria and arbovirus infections (Sow et al., 2016a). **Malaria was detected by high-grade fever for patients** exhibiting dual infections compared with single malaria attack (Sow et al., 2016b). Given the same clinical presentation, **it is difficult to** determine the pathogen responsible for the clinical signs and symptoms in the **co-current** infection (Sow et al., 2016a).

Besides, arboviral infection is tested only when malaria is tested negative, which set up the idea of misdiagnosing (Sow et al., 2016a). Thick blood smear and rapid diagnostic test for malaria diagnosis and reverse transcriptase- polymerase chain reaction, IgM **ELISA** and other viral infection detection was used for dengue virus, yellow fever virus, **Zika** virus, West Nile virus (arboviral infections) (Sow et al., 2016).

Different arboviruses circulated during malaria occurrence in this region of Africa (Sow et al., 2016a). During rainy seasons, there are periods of high malaria prevalence and hence some febrile illness of viral infection can be misdiagnosed as malaria (Sow et al., 2016).

Between 2000 and 2013, Ghana recorded 20,000 malaria-related deaths, including 2,506 in 2013. Additionally, approximately 50% of clinic visits in the country are due to febrile illnesses, such as typhoid fever and childhood pneumonia, which are often misdiagnosed as malaria, particularly during rainy seasons (Stoler & Awandare, 2016b). Dengue and other arboviral infections are potential causes of malaria-negative febrile illness in Ghana (Stoler & Awandare, 2016b). Most Rapid diagnostic test has been tested in sub-Saharan countries in Africa (Aidoo & Incardona, 2022). The use of tablet-based diagnostic algorithms without trained personnel can result in unnecessary costs, whereas the combination of both improves diagnostic quality. Co-exposure to dengue in lab-confirmed malaria patients complicates the diagnosis of febrile illness in Africa, particularly in malaria-negative cases (Bahaah et al., 2019).

Malaria, caused by *Plasmodium* parasites and transmitted by *Anopheles* mosquitoes, is an acute and potentially fatal disease that remains a significant global health threat (Hussein et al., 2020). As per the 2018 World Health Organization (WHO) statistics, around 228 million malaria cases were documented globally, leading to 405,000 deaths. Africa recorded the highest prevalence of cases and mortality rates (WHO, 2019).

Malaria stands as a prominent infectious ailment and represents the prevailing diagnosis for patients exhibiting fever symptoms (Asamoah et al., 2024). Malaria is widespread in Ghana, and approximately 40% of all outpatient visit involve children and pregnant women who are most vulnerable groups (Kawaguchi et al., 2022). In Ghana, *Anopheles gambiae* and *Anopheles funestus* serve as the predominant malaria vectors, with *Anopheles gambiae* increasingly prevalent in urbanized areas. Malaria is currently recognized as the most prevalent tropical disease and a leading cause of morbidity and mortality, especially in Africa (Bahaah et al., 2019). Malaria is prevalent among children under five years of age, who represent over two-thirds of all malaria related fatalities. In West Africa, more than 300 million individuals are at risk of malaria, with the highest vulnerability observed in young children and pregnant women (Bahaah et al., 2019). Many clinicians diagnose fever as malaria, resulting in overdiagnosis. This contributes to inefficiencies in healthcare systems, increased patient costs, prolonged hospital stays, and potentially higher mortality rates (Leslie et al., 2012).

In 2016, global reports indicated 216 million new cases of malaria and associated fatalities and 445,000 respectively (Yankson et al., 2019). Further more Rapid diagnostic test is very expensive and due to its inability to detect low parasitemia and persistence of positivity for up to 15 days post-treatment, relying solely on clinical signs and symptoms in over 90% of cases may lead to misdiagnosis (Paintsil et al., 2019).

A major challenge to the global control and eventual elimination of malaria is the prevalence of asymptomatic *P. falciparum* carriers, the presence and distribution of highly diverse *P. falciparum* isolates (including drug resistant parasites) (Wongsrichanalai & Sibley, 2013). Asymptomatic individuals serve as transmission reservoirs, as these individuals do not treat and clear their infection but rather remain infectious for the duration of parasite carriage (Abukari et al., 2019).

The five Plasmodium species known to infect humans are *Plasmodium falciparum*, *Plasmodium*

ovale, *Plasmodium malariae*, *Plasmodium vivax*, and *Plasmodium knowlesi*, but only the first three have been reported in Ghana (Owusu et al., 2017; Amoah et al., 2019). *Plasmodium falciparum* is the prevailing species (90-98%) with few *P. malariae* (2-9%) and *P. ovale* infections found (Owusu et al., 2017). ***Plasmodium falciparum* and *Plasmodium viva*** are primary parasite species that cause malaria in humans.

(Garrido-Cardenas et al., 2019). In 2015, malaria is responsible for approximately 8% of deaths in Ghanaian children under 5, with *Plasmodium falciparum* contributing to 44% of all reported outpatient cases in hospitals (Bahaah et al., 2019).

The *An. gambiae*, *An. arabiensis*, *An. melas*, *An. merus*, *An. funestus*, *An. quadrianulatus* and *A. bwanbae* are the seven species that vary in their ability to transmit malaria. *An. gambiae* is found alongside *An. funestus*, and both species compete in terms of their significance as malaria vectors (Dery et al., 2010; Anto et al., 2009).

In northern Ghana, *Anopheles funestus* is well-documented as a malaria vector, and in neighboring Burkina Faso, it is also recognized for its efficiency in transmitting malaria. However, *Anopheles gambiae* is more widely implicated in malaria transmission across West Africa, particularly in Ghana (Dery et al., 2010).

2.7 Dengue Diagnostic Challenges.

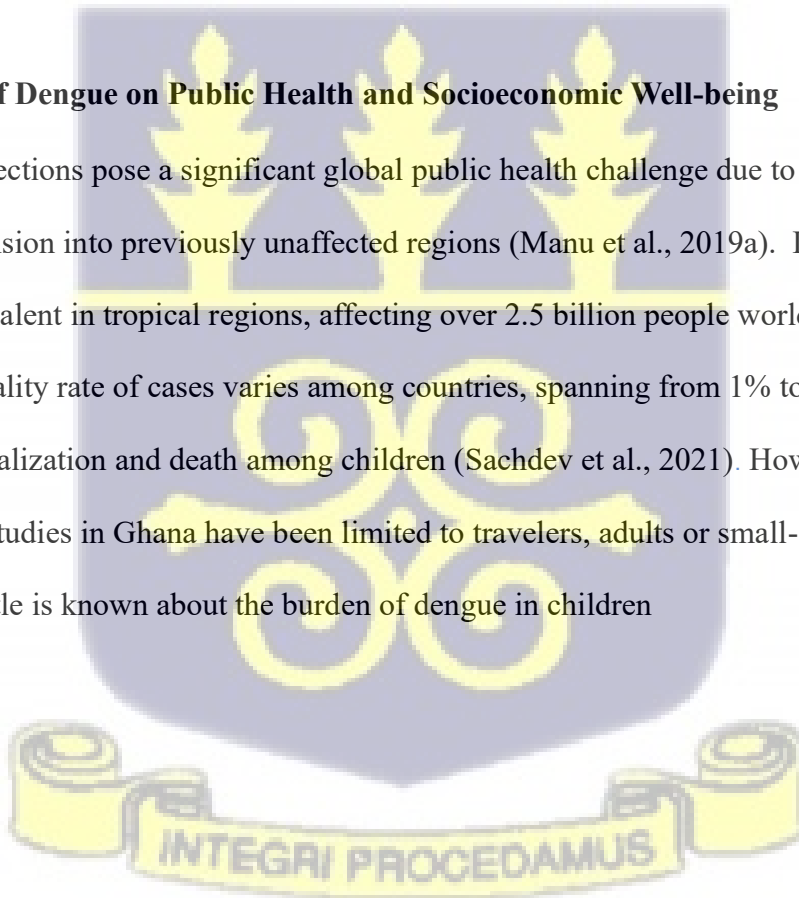
Diagnosing dengue fever clinically can pose difficulties, primarily contingent upon the stage of infection at which a patient seeks medical attention (Muller et al., 2017b). Dengue can exhibit symptoms include influenza, measles, Zika, chikungunya, yellow fever, and malaria at the early phases of clinical illness (Rahman, 2020). In areas endemic to flaviviruses like yellow fever and Japanese encephalitis, serological detection of DENV is challenging (Musso & Desprès, 2020).

This is as result of Antibody cross-reactivity results from shared epitopes on the flavivirus E protein (Muller et al., 2017b).

Due to cross-reactivity, utilizing antibodies against specific flaviviruses in DENV serology studies may yield false positive outcomes (Muller et al., 2017b). Therefore, to mitigate these erroneous positive outcomes, it is advisable to Combine IgM and IgG serology with the detection of the NS1 antigen (Marques et al., 2020; Muller et al., 2017b). **The absence of diagnostic facilities in many healthcare settings makes it challenging to accurately determine the true prevalence of such illnesses, leading to frequent underreporting of these diseases (Chipwaza et al., 2014b).**

2.8 Impact of Dengue on Public Health and Socioeconomic Well-being

Dengue viral infections pose a significant global public health challenge due to their epidemic nature and expansion into previously unaffected regions (Manu et al., 2019a). DF and DHF diseases are prevalent in tropical regions, affecting over 2.5 billion people worldwide (Sanyaolu, 2017). The mortality rate of cases varies among countries, spanning from 1% to less than 20% leading to hospitalization and death among children (Sachdev et al., 2021). However, despite this evidence, most studies in Ghana have been limited to travelers, adults or small-scale community surveys. Very little is known about the burden of dengue in children



CHAPTER THREE

3.1 METHODS

3.2 Study Site

The study was conducted in two health facilities within the Bono East Region of Ghana: Atebubu District Hospital and Kintampo Municipal Hospital. The Bono East Region, carved out of the former Brong Ahafo Region, shares borders with the Savannah Region to the north, the Bono Region to the west, the Ashanti Region to the south, and the Volta Lake to the east. Situated in Ghana's vegetative belt, the region enjoys consistent and favorable climatic conditions. The economy of the area is largely agricultural, with yam, beans, cashew, cocoyam, plantain, and maize as the primary crops, alongside fishing activities. Despite the agricultural and rural nature of the region, there is a notable absence of epidemiological and surveillance data on dengue. This lack of data necessitates a strategic selection of study sites within the region. The selection of the sites is based on geographical features and the historical presence of *Aedes* mosquitoes, the primary vectors of dengue transmission, as documented in studies (Amoa-Bosompem et al., 2020); (Appawu et al., 2006; Esena & Amoyaw, 2013; Suzuki et al., 2016).

Kintampo Municipal Hospital

Kintampo Municipal Hospital, established by the British colonial government, historically served the personnel of the Royal West African Frontier Force stationed in Kintampo. The hospital currently has a capacity of 125 beds and is equipped with key medical facilities and services. It plays a crucial role in providing healthcare to the population of the Kintampo Municipality and surrounding areas. The facility is staffed by qualified medical personnel, including a resident medical doctor, and has a broad range of healthcare services.

Atebubu District Hospital

Atebubu District Hospital serves as the primary healthcare facility for the Atebubu-Amantin District in Bono East. It is a key healthcare provider for the district and neighboring areas, positioned centrally within a network of four surrounding districts. The hospital has seven key departments: Records, Consultancy, Laboratory, X-ray, Pharmacy, Maternal and Child Health, and Ear, Nose, and Throat. With a daily outpatient attendance averaging 380 patients and a bed capacity of 60, the facility is critical to healthcare delivery in the region.

The study sites have been chosen for their accessibility to febrile patients and their central role in healthcare delivery in the Bono East Region. These facilities are well-positioned to provide insight into the prevalence of dengue and related arboviral infections due to their geographic and clinical significance.

3.3 Study design

This was a hospital based **cross**-sectional study. The study was carried out from January to March

3.4 Study Population

The study population was both **young males and females** in each of the study communities. Participants were recruited for the study after explaining the purpose of the study to them and consenting to the study. Each participant would be screened for dengue viral infection.

3.5 Inclusion criteria

The study populations include Individuals **aged one year to 17 years** who exhibit symptoms like a high fever (38°C or higher), chills, headaches, retro-orbital pain, muscle and joint pain, nausea, vomiting, rash, body aches, weakness, lethargy, malaise, loss of appetite, swollen lymph nodes,

and bleeding manifestations are eligible to participate. Participants who gave written informed consent. These populations were less than 18, and consent was sought from parents.

3.6 Exclusion Criteria

Individuals with other medical conditions or those who did not provide informed consent.

3.7 Sample size Calculation

A literature search revealed that the prevalence of dengue fever in neighboring countries ranges from 2.2% to 8.5%, depending on serological and PCR testing methods. Notably, in Burkina Faso, there was a significant increase in rates in 2023. According to WHO reports as of December 18,

2023, the country recorded a cumulative total of 146,878 suspected cases for the year, including 68,346 probable cases (positive rapid diagnostic tests) and 688 deaths among suspected cases, resulting in a case fatality rate of 0.5%.

Given the current rise in infection rates within the general population and the inadequate testing capacity, it is reasonable to assume that the prevalence of dengue fever could approximate 8.5%. This estimated effect size of 8.5% will be sufficient to detect cases at a 95% confidence level.

To determine the sample size needed for a hospital-based cross-sectional study with an estimated prevalence of 8.5%, the sample size calculation formula for a proportion will be employed. This approach ensures that the study is adequately powered to yield statistically significant results.

The formula is as follows: n

is the sample size.

Z is the Z score corresponding to the desired confidence level (for example, for a confidence level of 95%, $Z \approx 1.96$).

p

p is the estimated proportion in the population (8.5% in your case, $p = 0.085$).

E is the acceptable margin of error, expressed in decimals.

A margin of error of, for example, 5%, we can use these values to calculate the sample size.

Substituting these values into the formula, we obtain:

$$Z^2 \times p \times (1 - p) / E^2$$

$$n = 1.962(1.962) \times 0.085(1 - 0.085) / 0.05(0.05)$$

$$n \approx 119.4$$

A minimum of 119 subjects for your hospital-based cross-sectional study with an estimated proportion of 8.5%, a confidence level of 95%, and a margin of error of 5%.

3.8 Study procedure

The study was explained in detail to their parents who attended to the outpatient departments (OPD) at the selected hospitals, ensuring they fully understand the purpose, procedures, and potential benefits or risks of participation. Informed consent was obtained from all participants who voluntarily agree to be part of the study. Socio-demographic data was then collected using a well-structured questionnaire. The questionnaire also included questions designed to gather information on potential predictors of dengue viral infection, such as recent travel history, exposure

to mosquito bites, environmental conditions, and prior medical history. The comprehensive data collection assisted for a robust analysis of factors influencing dengue prevalence among febrile patients in the study population.

3.9 Sampling procedure.

A total enumeration technique was employed to select participants for the study. All febrile patients visiting the OPD of the selected hospitals (Atebubu District Hospital and Kintampo Municipal Hospital) during the study period were eligible and invited to participate. This approach ensured that every eligible febrile patient presenting to the OPD was given the opportunity to be included in the study, thereby maximizing the sample size and improving the representativeness of the findings.

3.10 Sample collection

A total of 237 blood samples was collected from participants at Atebubu District Hospital and Kintampo Municipal Hospital. **200 samples were collected from Atebubu Hospital and 37 samples were collected from Kintampo Hospital.** Approximately 5 mL of blood was collected into the EDTA tube. The blood samples were used for Dengue Rapid Diagnostic Testing (RDT) using the SD Biosline kit. Once collected, the blood samples were processed to separate the serum. Approximately 1 mL of serum was aliquoted into two separate vials for each participant. These vials were then temporarily stored at -20°C , ensuring the preservation of the samples for further testing and analysis (Manu *et al.*, 2019). The serological and Molecular test was done in Noguchi Memorial Institute for Medical Research.

3.11 Laboratory detection of Dengue Viral Infection

Diagnoses for dengue was confirmed by means of positive dengue-specific NS1 antigen and IgG antibodies RDTs and ELISA as well as positive RT-PCR and sequence to determine the circulating

serotype. All testing was carried out as per the manufacturers' instructions and the calculations and interpretations of the test results was according manufacturers' protocol.

3.11.1 The Dengue NS1 Antigen Rapid Diagnostic Tests (RDTs) Outcomes

The RDT for dengue NS1 antigen and IgG antibodies is a qualitative membrane-based immunoassay that detects dengue antibodies in whole blood, serum, or plasma. This assay consists of a dengue NS1 test in addition to a second test that detects both IgG and IgM antibodies. For detection of dengue NS1 antigen in whole blood, serum, or plasma, using the qualitative membrane-based immunoassay known as dengue NS1. Dengue antibody-conjugate in the test cassette enables the sample to react during testing. The Gold antibody conjugate binds to the dengue antigen in the sample, which then interacts with anti-dengue NS1 antibodies on the membrane. As the reagent moves, the antibody-antigen complex is captured, forming a pink line at the test region. The intensity of the line correlates with the antigen concentration, and the appearance of the line indicates a positive result.

3.11.2 Enzyme-linked Immunosorbent Assay

Serum samples were analyzed for Dengue- specific markers including NS1 antigen, IgM and IgG antibodies using the Anti-Dengue Virus IgM and IgG ELISA kits (SD Biosensor Ultra Dengue IgM and IgG). The capture IgM ELISA and indirect IgG ELISA are important techniques for surveillance, valued for their high sensitivity, simplicity, specificity, and practicality. In nonendemic regions, these methods are utilized in clinical surveillance of viral infections, with positive results indicating recent **infection** (Peeling et al., 2010).

3.11.3 NS1 Antigen Enzyme linked Immunosorbent Assay

SD Biosensor Dengue NS1 antigen Elisa method utilizes a microplate that has been pre-coated with monoclonal anti-dengue NS1 antibodies. During the initial incubation, dengue NS1 antigens in the patient's sample specifically bind to these antibodies, forming a complex. Subsequently, a monoclonal anti-dengue NS1 antibody conjugated with horseradish peroxidase (HRP) binds to the NS1 antigen, creating a "sandwich" structure. After a wash step to remove unbound components, the remaining enzyme activity in the wells is indicative of the NS1 antigen concentration in the sample. The enzyme activity is detected by adding a substrate solution (TMB, tetramethylbenzidine), which produces a color change. The color intensity is indicative of directly proportional to the amount of dengue NS1 antigen in the sample, thereby allowing for quantitative analysis of the antigen concentration.

3.11.3.1 Preparation of Reagent

Wash Buffer (20 times concentrated) was diluted 4 to 1 using distilled water before use. **125 μ l of wash buffer (20 times concentrated) with 25 μ l of distilled water.**

3.11.3.2 Test procedure.

Samples were diluted with a ratio of 4: 1 with sample buffer and mix well. **125 μ l of the sample** and 25 μ l of sample buffer. Incubate the mixture for at least 10 minutes at room temperature (15-25°C). Undiluted 125 μ l of positive and negative controls is dispensed into five respective wells (2 wells for positive control and 3 wells for negative controls). Then the microplate was sealed with adhesive plate film and incubated at 36-38°C for 1 hour.

Subsequently, the wells are washed with 350 μ l of diluted wash buffer and all liquid were aspirated from the wells. The wash buffer is left in each well for 4-5 seconds per washing cycle and then emptied. The liquid from the microplate were thoroughly disposed **off** tapping it on absorbent paper. Then 100 μ l of enzyme conjugate was added into each well. The microplate wells were

sealed with adhesive plate film and incubated at 36-38°C for 30 minutes. After incubation, the solution underwent a secondary washing. Following the incubation period, 100 µl of TMB substrate was introduced into each well of the microplate in a consistent order and incubate for 10 minutes and at a uniform speed as that of the stop solution. The contents were thoroughly mixed to halt the reaction. Within 30 minutes the absorbance value of each well using the microplate reader fitted with a 450nm filter were measured. If dual wavelength spectrophotometer is available and the reference filter between 620-650nm.

3.11.3.3 Interpretation of test result

The mean absorbance of the triplicates of negative control was calculated and the cut off factor (0.400) was added to the mean absorbances of the triplicates of the negative control to obtain the cut-off value.

The index value was calculated by dividing the sample absorbance by the cut-off value.

Index Value = $\frac{\text{Absorbance of sample}}{\text{Cut-off Value}}$

Cut-off Value.

Table 1. Interpretation of NS1 Test Results

Index Value	Result	Interpretation
Index value 0.8	Negative	No detectable NS1 antigen
index value 1.1	Equivocal	The samples should be retested
Index value 1.1	Positive	Presence of detectable NS1 antigen. It indicates primary dengue virus Infection.

3.11.4 Dengue IgG Enzyme linked Immunosorbent Assay

The SD Biosensor Ultra Dengue IgG ELISA test uses immunochromatography to detect IgG antibodies specific to the dengue virus in human serum, plasma, or whole blood samples, intended for in vitro diagnostic use. The microplate in the kit is pre-coated with monoclonal anti-human IgG antibodies. During incubation, patient IgG antibodies bind to these monoclonal antibodies, followed by binding to dengue antigens conjugated with horseradish peroxidase (HRP), forming a sandwich complex. After washing to remove unbound material, enzyme activity in the wells, proportional to anti-dengue IgG levels, is measured by adding a tetramethylbenzidine (TMB) substrate solution. The resulting color intensity correlates with the amount of anti-dengue IgG in the sample.

3.11.4.1 Preparation of reagent.

Wash Buffer (20 times concentrated) was diluted 1 to 99 using distilled water before use. 10 μ l to wash buffer (20 times concentrated) with 990l of distilled water.

3.11.4.2 Test Procedure

Samples were diluted with a 1:99 ratio using sample buffer and thoroughly mixed. A 100 μ L volume of undiluted positive and negative controls was added to the designated wells of the microplate. Then the microplate was sealed with adhesive film and incubated at 36-38°C for 1 hour.

Subsequently, the wells were washed five times with 350 μ l of diluted wash buffer, and all liquids were aspirated from the wells. The wash buffer was allowed to remain in each well for 4-5 seconds per washing cycle, and all liquid from the microplate was thoroughly disposed of. 100 μ l of diluted enzyme conjugate (HRP-labelled monoclonal anti-dengue-env with dengue antigen).

After incubation, the solution underwent a secondary washing. Following the incubation period, 100 μ l of TMB substrate was introduced into each well of the microplate in a consistent order and incubate for 10 minutes and at a uniform speed as that of the stop solution. The contents were

thoroughly mixed to halt the reaction. Within 30 minutes the absorbance value of each well using the microplate reader fitted with a 450nm filter were measured.

3.11.4.3 Interpretation of test result

The mean absorbance of the triplicates of negative control was calculated and the cut off factor (0.400) was added to the mean absorbances of the triplicates of the negative control to obtain the cut-off value.

The index value was calculated by dividing the sample absorbance by the cut-off value.

Index Value = $\frac{\text{Absorbance of sample}}{\text{Cut-off Value}}$

Cut-off Value.

Table 2. Interpretation of Dengue IgG Test Results

Index Value	Result	Interpretation
Index value 0.8	Negative	No detectable NS1 antigen
index value 1.1	Equivocal	The samples should be retested
Index value 1.1	Positive	Presence of detectable NS1 antigen. It indicates primary dengue virus Infection.

3.11.4 Molecular diagnosis

The molecular diagnosis was performed at virology laboratory in Noguchi Memorial institute for Medical Research University of Ghana Legon.

3.11.5 RNA Extraction

RNA extraction was performed using the QIAamp Viral RNA Mini Kit (250) (Qiagen, Hilden, Germany) following the manufacturer's protocol on 140 μ L of serum. 140 μ L of serum was added to the 560 μ L of lysis buffer vortex and incubated at a room temperature for 10 minutes. 500 μ L of absolute cold ethanol was added to the solution and vortex. Binding was achieved by centrifuging the solution in a spin column at 8000rpm for 1 minute.

500 μ L of washing buffer 1 was added and centrifuged at 8000rpm for 1 minute. Wash buffer 2 was added to the solution and centrifuged at 14000rpm for 3 minutes. The solution was dried spin at 14000rpm for 1 minute. 60 μ L elution buffer was added to extract the RNA. In cases where PCR was to be conducted within a 24-hour period, we stored the extracted RNA at -80°C. Otherwise, we stored it at -20°C.

3.11.5.1 Real Time PCR Analysis.

Real Time PCR was performed at Noguchi Memorial Institute for Medical Research. Dengue RTPCR assay was used for amplification of the DENV according to the manufactures protocol. Nucleic acid amplification assays were employed to detect the presence of arboviral nucleic acids. The assays utilized included the CDC Trioplex Real-time RT-PCR (Trioplex rRT-PCR) performed with the Agpath-ID One-Step RT-PCR kit (Lot number: 2007259) for identifying Dengue virus. Additionally, a TaqMan-based real-time reverse transcription polymerase chain reaction (rRT-PCR) assay, as described by Johnson *et al.* 2005, was used to identify all four DENV serotypes in the samples.

Total volume of reaction was 375 μ L consisting of 12.5 μ L of nuclease free water, 312.5 μ L of reaction buffer, 12.5 μ L of DENV primer mix, 12.5 μ L of enzyme. The reaction was vortex and dispense 5 μ L of the reaction into 96 well reaction plate. 2.5 μ L of the extract was added to the master mix in the 96 well. The 96 well reaction plate was covered with optical adhesive film. The

96 -well reaction plate was centrifuged for 30 seconds. Reverse transcription was carried out for 20 mins at 55°C. Cycling step with denaturation of 2mins at 95°C, annealing of 15s at 55 °C for 45 cycles. The amplification including the extension was carried out for 15s at 72°C. PCR machine 700 FAST (Applied Biosystem 7500 FAST). We included negative and positive controls in these rRT-PCR assays and Agpath-ID One-step RT-PCR users guide report for results interpretation.

3.11.5.2 Conventional PCR

Positive NS1 antigen was confirmed with conventional PCR. Qiagen one step including DUS and DUC primers (lot number: 172027512) was used as an enzyme for the preparation of the master mix. Total volume of reaction was 500µl consisting of 200µl of nuclease free water, 62.5µl of DUS primer, 62.5µl of DUC primer, 25µl of Deoxyribonucleoside triphosphate(dNTP), 125µl of reaction Buffer and 25µl of enzyme mix. 5µl was dispensed in 96 well. 2.5µl of the extract is dispensed in the 96 well and covered with adhesive film. The 96 well reaction plate was centrifuged for 30 seconds. The reaction was set at 1hour,30 minutes to 2 hours. Initial denaturation for 2-5 minutes at 94-98°C. Thermal cycling phase involving DNA denaturation of 15s-30s at 95°C -98C, annealing of 15s -60s at 50-65°C for 45 cycles. Extension of 30s -60s at 72°C. The amplification including the extension was carried out for 5-10minutes at 72°C. Then cooling at 4 °C. PCR machine 700 FAST (Applied Biosystem 7500 FAST). We included negative and positive controls in these rRT-PCR assays to get the amplicons.

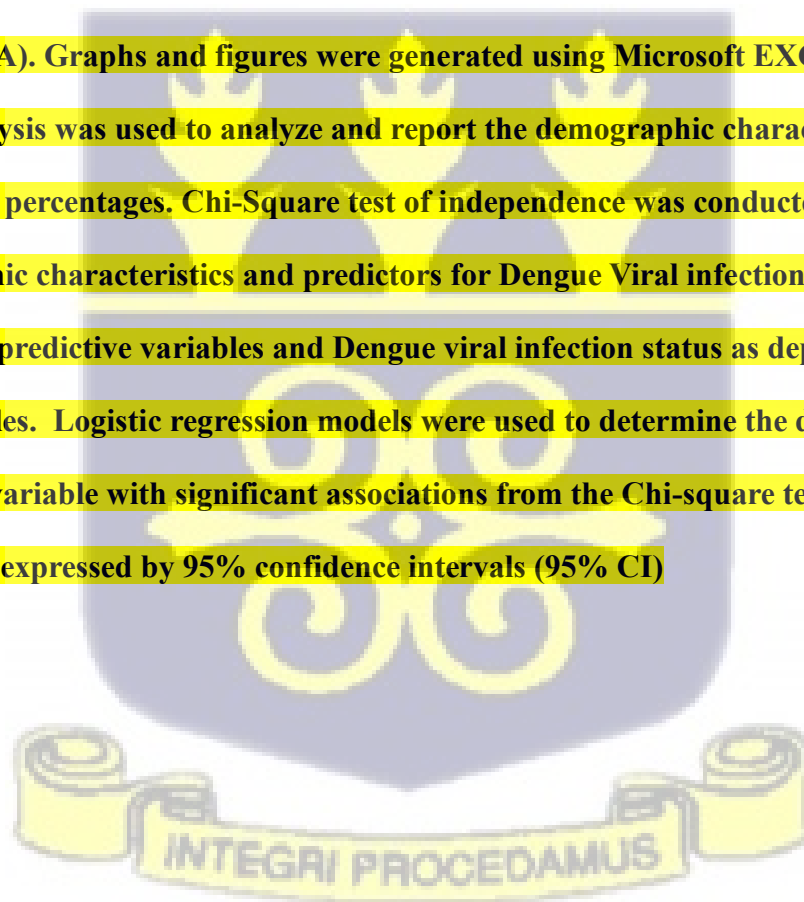
3.11.5.3 GEL Preparation

2% of gel preparation was made. 2g of agarose powder was weighed and 50ml of Tris acetate EDTA (TAE buffer) was measured using measuring cylinder. Dissolve the agarose powder and the TAE buffer. Microwave the dissolved agarose and the TAE buffer for 2mins to a clear solution. The tray the comb was fixed. 2µl of gel red was pipetted into dissolved solution and swell for

homogeneity. The dissolved solution was poured into the tray and waited for 15-30 minutes to solidify. The combs were removed. The remaining TAE buffer was poured into the tray and the loading dye as well as the amplicon were placed on parafilm. The mixture of the loading dye and the amplicons were load into the ladder in the gel. Positive control, DC-24-409 was used. The gel was placed in gel tank and plug for 40 minutes. The bands of the RNA visualized with gel doc system.

3.12 Statistical Analysis.

Data entry and cleaning was carried out using Microsoft EXCEL. Statistical analysis (descriptive, univariate and multivariable) was conducted in STATA version 17.1 (Stata Corp Texas, USA). Graphs and figures were generated using Microsoft EXCEL. The descriptive analysis was used to analyze and report the demographic characteristics in frequencies and percentages. Chi-Square test of independence was conducted using sociodemographic characteristics and predictors for Dengue Viral infection as independent or predictive variables and Dengue viral infection status as dependent or outcome variables. Logistic regression models were used to determine the direction of association for variable with significant associations from the Chi-square test at a p-Value of 0.05 and was expressed by 95% confidence intervals (95% CI)



CHAPTER FOUR

4.0 RESULTS.

4.1 Socio-demographic characteristics of the Population

Two hundred and thirty-seven (237) febrile pediatric patients were enrolled in this study comprising 135 males (56.96%). Majority of participants were between 1 and 3 years old, 17.72% were between 4 and 5 years old, 33.33% were aged 6 to 12 years, and 7.17% were between 13 and 17 years old. Most participants identified as Christians (68.35%), while 31.65% were Muslims.

Details of the Dengue test is presented in Table 3.

Table 3. Prevalence of dengue antigen and antibodies in study participants

<u>Characteristics</u>	<u>Frequency (N)</u>	<u>NS1 Antigen (%)</u>	<u>IgG (%)</u>	<u>PCR (%)</u>
Sex	237	5.4 % (13)	7.5% (18)	2.1% (5)
Male	135	(5.93)	(11.11)	(2.22)
Female	102	(4.9)	(2.94)	(1.96)
<i>p</i> -value		0.73	0.02	0.89
Age (Years)				
- 3	99	(8.08)	(4.04)	(2.02)
- 5	42	(7.14)	(16.67)	(4.76)
- 12	79	(2.53)	(6.33)	(1.27)
- 17	17	(0)	(11.76)	(0)
<i>p</i> -value		0.28	0.06	0.56
Religion				
Christian	162	(3.7)	(6.79)	(1.85)
Muslim	75	(9.33)	(9.33)	(2.67)
<i>p</i> -value		0.08	0.49	0.69
Education				
No Education	11	(0)	(0)	(0)
Primary	90	(5.56)	(11.11)	(3.33)
JHS	10	(0)	(20)	(0)
SHS	2	(0)	(0)	(0)
Unspecify	124	(6.45)	(4.84)	(1.61)
<i>p</i> -value		0.72	0.48	0.86

The overall prevalence of dengue NS1 antigen, IgG antibodies, and PCR-confirmed dengue virus was 5.4%, 7.5%, and 2.1%, respectively. **The prevalence of Atebubu hospital is 6.5% and that of Kintampo hospital is 0.**

4.2 Gender Difference and Prevalence of Dengue Infection.

The prevalence of NS1 antigen was 5.93% in males and 4.9% in females. IgG antibody prevalence was 11.11% in males and 2.94% in females. There was a difference in IgG seropositivity among participants with different sex ($p=0.02$).

4.3 Prevalence of Dengue in Different Age Groups.

Among different age groups, the prevalence of NS1 antigen was highest in children aged 13(8.08%) while IgG antibody prevalence was highest in children aged 4-5 years (16.67%). A marginally significant difference in seropositivity was observed across age groups.

4.4 Prevalence of Dengue Infection Among the Religion

By religion, Muslims recorded the highest prevalence of NS1 antigen (9.33%). There was no significant difference in IgG seropositivity among participants of different religions. However, a marginally significant difference was observed in NS1 antigen prevalence across religious groups.

4.5. Dengue Virus Serotyping

Serotyping using PCR identified DENV-1 as the predominant strain, with all PCR-positive samples classified as serotype 1. Overall, 2.1% of participants were PCR positive, with cycle threshold (CT) values below 30 for NS1-positive samples, indicating high viral loads. PCR confirmed dengue infection was slightly higher in males (2.22%) than females (1.96%).

By age, children aged 4-5 years had the highest prevalence (4.76%). Muslims recorded the highest prevalence by religion (2.67%), while primary school children showed the highest prevalence by education level (3.33%). No statically differences were observed by sex, age, religion or education.

4.6 Educational Status and Dengue Prevalence

There was no significant association between dengue infection and educational status. Primary school children recorded the highest prevalence of NS1 antigen, while IgG seropositivity was highest among primary and junior high school participants. Notably, Middle/JHS students had the highest IgG prevalence (20%) compared to other educational groups.

2. Predictors of dengue viral infection among febrile patients in Bono East of Ghana.

Table 4. Predictors for dengue viral infection among febrile patients

Predictor	Frequency (n)	Positive	Positivity rate (%)	P-Value	χ^2
Travel History	26	0	0	1.69	0.19
Flower pots	21	0	0	1.34	0.25
anything that collect water	57	6	10.53	3.68	0.06
store water in containers at home?	205	10	4.88	1.08	0.29
High fever	226	13	5.75	0.67	0.41
Headache	156	9	5.77	0.07	0.79
Pain (Body and joint)	41	0	0	2.88	0.09
Eye pain (Retro-orbital pain)	8	0	0	0.48	0.49
Nausea/vomiting	89	5	5.62	0.005	0.95
Bleeding nose/ bleeding gum	6	1	16.67	1.48	0.22
Bloody urine	6	0	0	0.36	0.55
Rash	14	2	14.29	2.22	0.14
Refused meals	88	8	9.09	3.51	0.06
General fatigue	56	6	10.71	3.87	0.05
Cough	35	4	11.43	2.79	0.09
Stomach discomfort/disorder	74	7	9.46	3.28	0.07

4.7 Environmental and Behavioral Risk Factors

Exposure to water-collecting items was associated with dengue prevalence of 10.53%, though this was not statistically significant. Participants who stored water in containers recorded a prevalence of 4.88%, with no significant association observed. None of the 26 participants with travel history and none of the 21 participants with flower pots tested positive for dengue infection. A prevalence of 9.09% was observed among participants who reported refusal of meals, and this showed a marginally significant association with dengue infection.

4.8 Association Between Dengue Infection and Symptoms

Fever was the most frequently reported symptom, occurring in 13% of participants. Headache was reported by 156 participants, of whom 9 (5.77%) tested positive for dengue infection. Among the 41 participants with body and joint pains, none tested positive. A prevalence of 14.29% was observed among participants who presented with rash. Symptoms such as body pains, cough, and gastrointestinal disorders showed marginal associations with dengue infection. A statistically significant association was observed between dengue infection and general fatigue ($p = 0.05$).

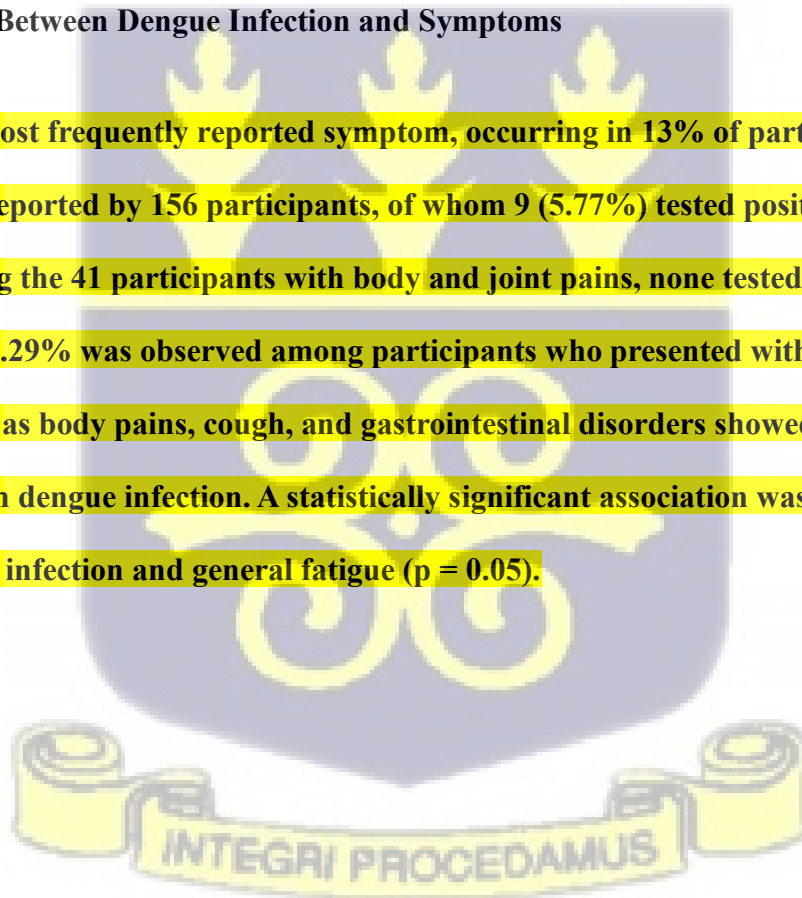
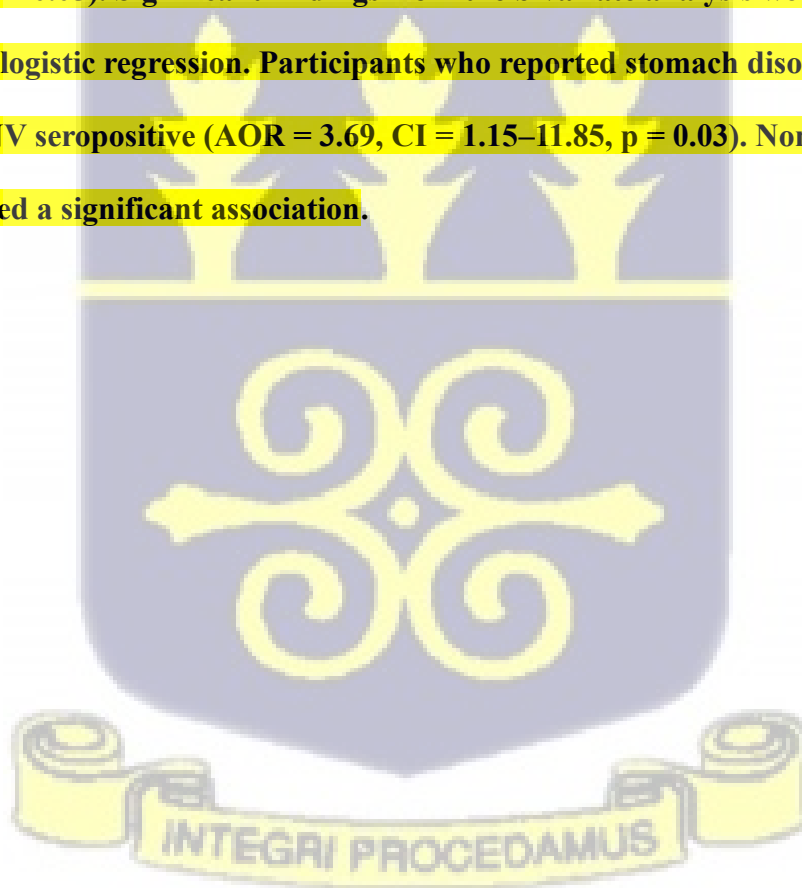


Table 5. Multivariate association between predictors and dengue infection.

Variable	OR (95% CI)	p-Value	AOR (95% CI)	p-Value	AOR (95% CI)	p-Value
General Fatigue	2.98 (95 9.28)	0.06	3.69 (1.15 -11.85)	0.03	3.66 (1.14 11.78)	0.03

In the bivariate analysis, only general fatigue was a significant predictor of DENV seropositivity ($p = 0.05$). Significant findings from the bivariate analysis were further examined using logistic regression. Participants who reported stomach disorders were less likely to be DENV seropositive (AOR = 3.69, CI = 1.15–11.85, $p = 0.03$). None of the other predictors showed a significant association.



CHAPTER FIVE

5.1 DISCUSSION

Dengue fever present a major public health concern in tropical and subtropical areas, notably prevalent in Sub-Saharan Africa (Aniakwaa-Bonsu et al., 2021a). While understanding the global or national prevalence of dengue is crucial, assessing local infection rates is equally vital.

This study aimed to determine the prevalence of dengue infection among febrile pediatric patients in selected hospitals in Bono East. The investigation utilized dengue NS1 Ag ELISA for detecting NS1 antigen and PCR for confirmation, alongside dengue IgG capture ELISA for antibody detection.

The study revealed 5.4% prevalence of NS1 antigen, indicating an active dengue infection in the studied population surpassing previous findings in Ghana (Kofi Bonney et al., 2018b; Ofori Appiah et al., 2018a). Moreover, both studies reported lower prevalence rate (2.67% and 2.5 % respectively) of NS1 antigen in Ghana. **Additionally, prevalence rate in our study surpassed the 4.2% prevalence of NS1Ag reported in previous findings in Burkina Faso (Donatien et al., 2023). On the contrary, a study by reported no prevalence of NS1 antigen in Ghana (Manu et al., 2019b).** PCR analysis revealed a higher prevalence (2.1%) of dengue virus, predominantly serotype 1 (DENV-1), in the Bono East population. Factors contributing to this higher prevalence include favorable climatic conditions for Aedes mosquitoes and conducive transmission environments (Amodia-Bosompem et al., 2021).

Some of the study patients (7.5%) were reactive for dengue IgG. **Similar study conducted in Ghana reported 3.6% of IgG of dengue in Ghana (Ofori-Appiah et al., 2018b).** The difference

in prevalence between **this study and previous study was due the reason that most of the febrile patients have been previously infected and recovered (Ofosu-Appiah et al., 2018b).**

Furthermore, serotype-specific identified by PCR analysis revealed higher prevalence (2.1%) of dengue virus, in Bono East population, with all cases being classified as serotype 1(DENV-1) This could be as a result of the presence of dengue virus within Bono east region. In contrast, previous study reported the low prevalence (0.3%) of DENV-1, DENV-2 and DENV3 in all the region in Ghana from 2021-2023 **(Bonney et al., 2024).** The prevalence of PCR confirmed dengue virus was lower than that of the prevalence of NS1 antigen. This is attributed to high levels of circulating dengue NS1 antigen in the blood of the infected individuals. **Similarly, study recorded lower prevalence of confirmed PCR-dengue virus than the prevalence of NS1 antigen (64.89%) (Iqbal et al., 2023). Similar study detected higher NS1 antigen (80.9%) prevalence compared to dengue virus by PCR (Anand et al., 2016).** Also, similarities in the epitope of the nonstructural protein among flavivirus could lead to false positive results as a result of cross reactivity (Muller et al., 2017c). However, NS1 antigen can still be detected in dengue cases until day 12 postsymptoms onset, but PCR is 'highly sensitive and detect the specific dengue viral RNA at the early stage of the infection (Fisher et al., 2023).

5.2 Gender Differences in Prevalence

In this study, males were mostly infected with dengue NS1 antigen (5.93%) compared to females (4.9%), this difference was not statistically significant ($P= 0.73$). This indicates males were more active in outdoors games than females. **Similarly, a study reported higher prevalence of acute dengue infection in males in Pakistan (Zohra et al., 2024). Similar study conducted in India recorded high prevalence of dengue cases in males (64%) than females (Kumar et al., 2020) .**

Similar study also conducted in India reported high prevalence of dengue infection in males than females (3.4:1) (Mishra et al., 2016). In contrast, recorded high prevalence of dengue infection in females than males (Tchuandom et al., 2019). The difference in the prevalence between this study to that of the studies conducted in India and Pakistan is due to the reason that most parents of febrile pediatric patients disagreed for blood samples to be taken and this contributed to low prevalence of dengue Infection reported.

However, dengue immunoglobulin (G) positivity was significantly higher in males (11.11%) than in females (2.94%) ($p=0.02$). This could be as a result of different level of exposure to the infection due to environmental activities or immune response. **However, similar study revealed high prevalence of dengue antibody (46%) in males than that of females (S. Ahmed et al., 2015a).** The shifts in seroprevalence of dengue among males and females are linked to differences in virus exposure (Aniakwaa-Bonsu et al., 2021b). **In contrast detected high seropositivity of dengue IgG in females than males (Pratt et al., 2024).**

5.3 Age-based Trends of dengue Infection.

Age is one of the risks factors of dengue Infection. Dengue impacts individuals across all age groups, with the greatest burden observed among children, adolescents, and young adults. particularly in infants (Ghosh et al., 2025). Children aged 1-3 years (8.08%) were mostly affected with dengue NS1 antigen whereas no cases were detected in other age groups. This may be as a result of spending significant time at home or nearby areas with poor water storage and sanitation practices, which serves as a breeding site for mosquitoes. This may also be attributed to their developing immune system that may not effectively control the viral replication leading to higher production of NS1 antigen leading to the acute phase of the infection. **A similar study by in India**

recorded high positivity of NS1 antigen (69.8%) in children aged 1-5 years (T. Ahmed et al., 2023).

In this study, children aged 4-5 years and other older children reported high seropositivity for dengue IgG, this may indicate that they have developed immunity from past exposure. However, the association between age and dengue infection was not statistically significant. **On the contrary, other studies recorded high dengue seropositivity for IgG among age groups greater than 5 years which 61 % of dengue positive IgG in children aged more than 10 years as well as Mohsin et al., 2016 recorded 65.2 % of dengue positive IgG in children aged 5 to 20 years (S. Ahmed et al., 2015b; Mohsin et al., 2016) .**

5.4 Educational level and dengue Infection

This study however revealed no significant association between dengue infection and educational status. Primary school children recorded high prevalence of dengue NS1 antigen, and dengue IgG. This could be due to high exposure to in social environment. Primary school children spend a significant portion of their time in school environment where mosquito exposure is heightened. Many school grounds may have poorly managed areas with stagnant water, inadequate drainage or vegetation that can serve as breeding grounds for *Aedes* mosquitoes and children's natural tendency to play outdoor further increases their chance to be bitten. This led to exposure to virus and also contributed to a steady accumulation of immunity over time. **Similarly, a study reported high seroprevalence (5.8%) of dengue viral infection among school children (Endy et al., 2002). A related study conducted in Colombia reported high seroprevalence from 53.8% to 64.6% for dengue IgG in school-aged children from 2010 to 2012 (Restrepo et al., 2012).**

Additionally, reported high seroprevalence (34.1%) of dengue IgG in school children (Malavige et al., 2006). The seroprevalence of dengue IgG among the primary and Junior high school children was lower than that of who reported 71% of primary school children (Tuntaprasart et al., 2003).

5.5 Environmental and Behavioral Predictors for dengue Infection

The second objective of the study was to determine the predictors for dengue viral infection among febrile pediatric in the two hospitals in Bono East. Dengue infections is frequent in Bono east region which is part of the forest zone of Ghana (Owusu-Asenso et al., 2022). Indeed, studies conducted previously reported number of dengue cases in Kintampo (Amoako et al., 2018b; Stoler et al., 2014).

Few febrile children were exposed to anything that collects water, 10.53% were positive for dengue infection. **This could be attributed to high chance of breeding sites for mosquitoes which consequently, lead to dengue reported that the more used containers collect water, the more aedes mosquitoes lay their eggs and breed which increases the risk of dengue hemorrhagic fever (Yulianti & Hidayani, 2022).** Additionally, Majority of the study febrile pediatric population (205) stay in homes that store water in containers, 4.88% were positive for dengue NS1 antigen but was not significant. This could be as a result of an exposure to the bites of Aedes mosquitoes due to uncovered or improperly storage of water which support the breeding of mosquitoes. Also, this could be due to behavioral factors which plays a role in dengue transmission. **A previous study reported that effective lids reduce the transmission of zika and aedes mosquito-borne diseases (Pinchoff et al., 2021).**

5.6 Association of Dengue Infection and Symptoms

Majority of the febrile pediatric population (226) had fever but most of these febrile pediatric population was positive for dengue infection. The presence of fever in these children further confirms that they were in the viremia stage, during which the virus was actively replicating. This also suggest that many of the febrile children were recently exposed to the bites of the Aedes mosquitoes. **Similarly, study recorded 514 cases clinically diagnosed as dengue infection on sites, 100(19.5%) were identified by the reference lab as nondengue (Made Susila Utama et al., 2019). Similar study conducted recorded 11.4% of the febrile children positive for Dengue NS1 antigen had fever (Capeding et al., 2013).**

The study recorded febrile children with body and joint pains but none were positive for dengue NS1 antigen. This could be attributed to other infection which could be responsible for body and joint pains. **In contrast, previous study in India reported 13.48% of children with body and joint pains associated to dengue infection (Shultana et al., 2019). Similarly, a study reported that less than 30 to 50% of the affected children with Chikungunya exhibit body and joint pains (Ritz et al., 2015).**

The study revealed few febrile children refused meals and 9.09% were positive with dengue infection. There was a marginal significant difference ($p=0.06$). This may be as a result of nausea, abdominal pains and vomiting which lead to loss of appetite. **A similar study conducted in Bangladesh reported 14% of dengue infected children with decreased in appetite (Alma et al., 2024) .**

Few febrile children exhibited general fatigue and 10.7% were positive for dengue infection. In this study, dengue infection was significantly associated to general fatigue ($P=0.05$), this as a result of severe muscle pains, fluid loss and systemic inflammation response triggered by cytokines

release which contribute to metabolic disruption and energy depletion due to dengue infection. **A similar study reported that fatigue was recorded in dengue infected infants Colombia (Flórez et al., 2024). In contrast, a previous study reported that out of the 260 adults recruited, 158 had dengue and out of these 32% had fatigue at 2 months which was significantly associated to severe dengue ($p=0.024$) (Sigera et al., 2021).**

Furthermore, the study indicated few febrile children had cough and 11.43 % were positive for dengue infection. This could be as a result of respiratory complication due to severe dengue. The severity of dengue can cause complication in the lungs leading to respiratory symptoms such as cough. **A similar study found that 31.9% of children experienced cough due to dengue infection, which is consistent with similar findings which reported that 22% of children experienced cough due to dengue infection (Nusrat et al., 2024; Dash et al., 2021).**

This study found that gastrointestinal disorder was common in few febrile children positive with dengue infection. This may be due to dengue virus primarily targeting the vascular endothelium, including the thin layer cells of the gastrointestinal tracts. The virus interacts with endothelial cells, disrupting their integrity and leading to increased permeability of blood vessels walls. This may cause symptoms such as diarrhea and vomiting. **Similar studies reported that infected children with dengue presented common symptom like gastrointestinal disorder (M. A. S. Khan et al., 2021; Akram et al., 2023). This study recorded a lower prevalence of infected dengue children (9.6%) as compared to previous study (Akram et al., 2023)**

CHAPTER SIX

6.1 CONCLUSION

This study aimed to assess dengue viral infection among febrile pediatric patients in Bono East, addressing gaps in local dengue epidemiology. The findings underscore the prevalence of dengue antigen and antibodies in the region, emphasizing the need for routine diagnostics in healthcare settings. While sociodemographic parameters did not significantly correlate with dengue infection, general fatigue emerged as a notable symptom associated with dengue.

The study contributes to understanding how environmental and socioeconomic factors influence dengue spread in Bono East. These findings serve as a baseline for future comparative studies and advocate for enhanced diagnostic capabilities to facilitate early detection. Public health campaigns informed by these data can improve awareness and prevention efforts, potentially reducing disease burden through targeted interventions.



7.1 RECOMMENDATION

To improve the detection and management of dengue infection in the Bono East region, the following recommendations are proposed:

1. Enhancing Diagnostic Testing:

- Dengue ELISA IgM testing was not conducted in this study. Future research should include IgM testing to detect current dengue infections and provide a more comprehensive epidemiological assessment.
- To minimize bias in results, all NS1 antigen-negative samples should be subjected to PCR testing to confirm their dengue status. This will ensure more accurate prevalence estimates.

2. Public Awareness and Education

- Community education programs should be implemented to enhance awareness about dengue transmission, symptoms, and prevention methods.
- Public health campaigns should emphasize proper water storage, elimination of mosquito breeding sites, and personal protective measures such as the use of insecticide-treated bed nets and repellents.

3. Strengthening Healthcare Services

- Healthcare facilities should be equipped with essential diagnostic tools, including rapid tests, ELISA, and PCR testing, to improve early detection and timely management of dengue cases.
- Adequate training should be provided to healthcare professionals on dengue diagnosis, treatment, and outbreak management.

4. Dengue Vaccine Implementation

- The effectiveness of dengue vaccination depends on public acceptance. Community engagement programs should be introduced to assess knowledge, attitudes, and perceptions regarding the dengue vaccine.
- Governments and health agencies should work together to facilitate the introduction and accessibility of dengue vaccines in high-risk areas.

By implementing these recommendations, dengue prevention and control efforts in the Bono East region can be significantly improved, reducing disease burden and safeguarding public health.



8.0 CHAPTER EIGHT

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9.0 APPENDICES

9.1 APPENDIX 1: GHANA HEALTH SERVICE ETHICAL CLEARANCE

GHANA HEALTH SERVICE ETHICS REVIEW COMMITTEE

In case of reply the number and date of this Letter should be quoted

My Ref: ghs/rdd/erc-admin/ren/app/23
Your Ref. No.


Your Health. Our Commitment

Research & Development Division
Ghana Health Service
P. O. Box MB 190
Accra.
Digital Address: GA-050-3303
Tel: +233-0302-960628
Fax + 233-0302-685424
Mob + 233-050-3539896
Email: ethics.research@ghs.gov.gh
6th May, 2024

Francisca Zigah
Ghana Infectious Diseases Centre
Ghana Health Service
C/O P. O. Box WY 1895
Kwabenya, Accra

RE: REQUEST FOR RENEWAL OF ETHICAL APPROVAL

Reference is made to your letter on the above subject matter.

Please be informed that the Ghana Health Services Ethics Review Committee has reviewed the request and has given approval for renewal of the ERC letter.

GHS-ERC Number	GHS-ERC: 007/12/22
Study Title	Dengue Seroprevalence Study in Three Selected Regions Across Three Ecological Zones in Ghana
Effective Date of Renewal	6 th May, 2024
Expiry Date	23 rd January, 2025
GHS-ERC Decision	Renewal Approved

The following applies:

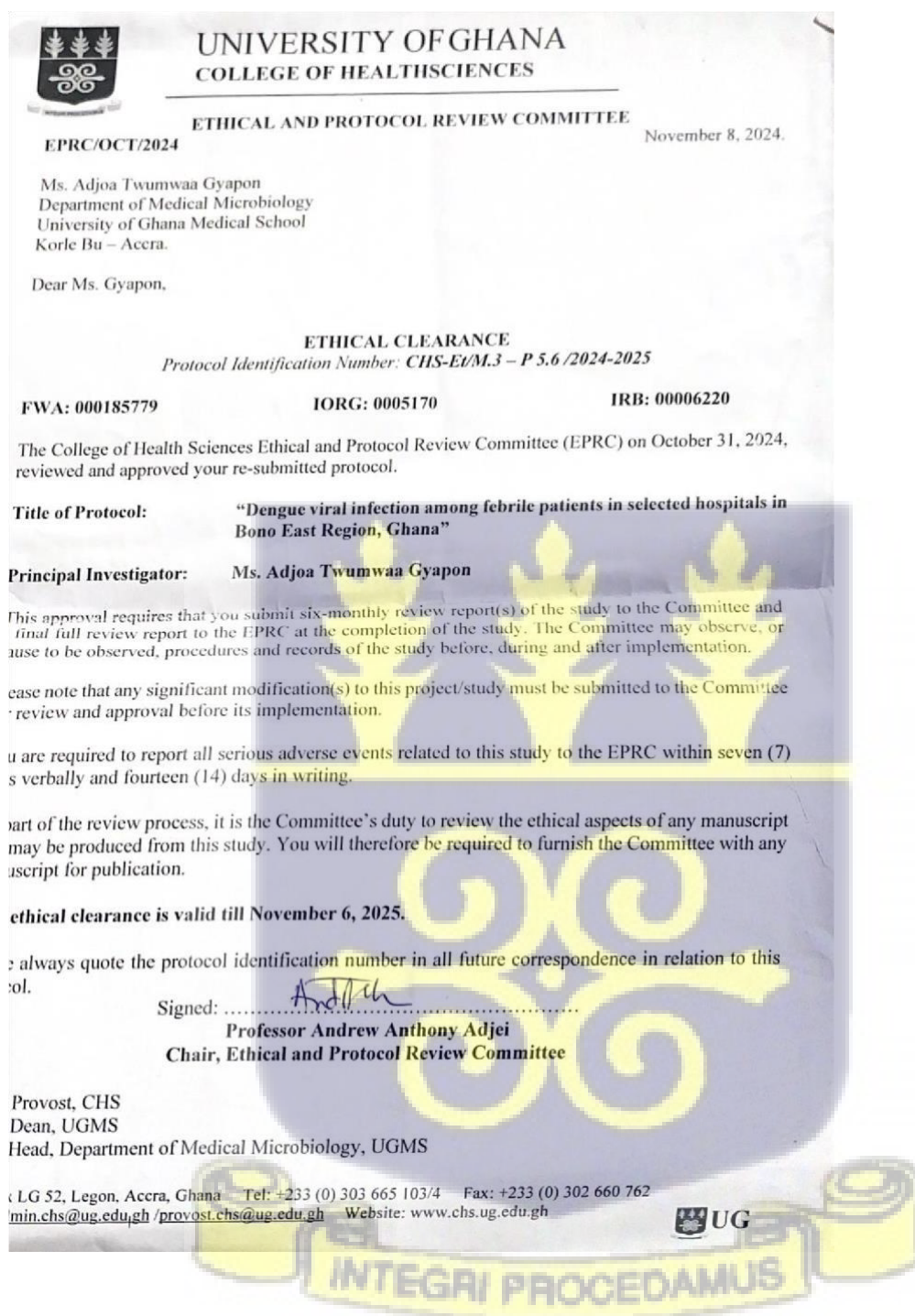
- Submission of yearly progress report of the study to the Ethics Review Committee (ERC).
- Renewal of ethical approval if the study lasts for more than 12 months.
- Reporting of all serious adverse events related to this study to the ERC within three days verbally and seven days in writing.
- Submission of a final report after completion of the study.
- Informing the ERC if study is discontinued and reasons why.
- Informing the ERC and your sponsor (where applicable) before any publication of the research findings.

1


INTEGRAI PROCEDAMUS

 CamScanner

APPENDIX 2: UNIVERSITY OF GHANA ETHICAL CLEARANCE



The image shows a formal ethical clearance certificate from the University of Ghana, College of Health Sciences. The document is dated November 8, 2024, and is addressed to Ms. Adjoa Twumwaa Gyapon. It grants ethical clearance for a study titled "Dengue viral infection among febrile patients in selected hospitals in Bono East Region, Ghana". The certificate includes the protocol identification number CHS-Et/M.3 - P 5.6 /2024-2025, FWA number 000185779, IORG number 0005170, and IRB number 00006220. It also states that the committee reviewed and approved the re-submitted protocol on October 31, 2024. The certificate is signed by Professor Andrew Anthony Adjei, Chair of the Ethical and Protocol Review Committee. The document features the University of Ghana logo and the motto "INTEGRA PROCEDAMUS" at the bottom.

UNIVERSITY OF GHANA
COLLEGE OF HEALTHSCIENCES

ETHICAL AND PROTOCOL REVIEW COMMITTEE

EPRC/OCT/2024 November 8, 2024.

Ms. Adjoa Twumwaa Gyapon
Department of Medical Microbiology
University of Ghana Medical School
Korle Bu – Accra.

Dear Ms. Gyapon,

ETHICAL CLEARANCE
Protocol Identification Number: *CHS-Et/M.3 – P 5.6 /2024-2025*

FWA: 000185779 IORG: 0005170 IRB: 00006220

The College of Health Sciences Ethical and Protocol Review Committee (EPRC) on October 31, 2024, reviewed and approved your re-submitted protocol.

Title of Protocol: “Dengue viral infection among febrile patients in selected hospitals in Bono East Region, Ghana”

Principal Investigator: Ms. Adjoa Twumwaa Gyapon

This approval requires that you submit six-monthly review report(s) of the study to the Committee and final full review report to the EPRC at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study before, during and after implementation.

Please note that any significant modification(s) to this project/study must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the EPRC within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee’s duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid till November 6, 2025.

You must always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed:
Professor Andrew Anthony Adjei
Chair, Ethical and Protocol Review Committee

Provost, CHS
Dean, UGMS
Head, Department of Medical Microbiology, UGMS

Plot LG 52, Legon, Accra, Ghana Tel: +233 (0) 303 665 103/4 Fax: +233 (0) 302 660 762
Email: min.chs@ug.edu.gh / provost.chs@ug.edu.gh Website: www.chs.ug.edu.gh

UG
INTEGRA PROCEDAMUS

9.2 APPENDIX 3- CONTROLS USED IN REAL TIME PCR ANALYSIS.

POSITIVE CONTROL

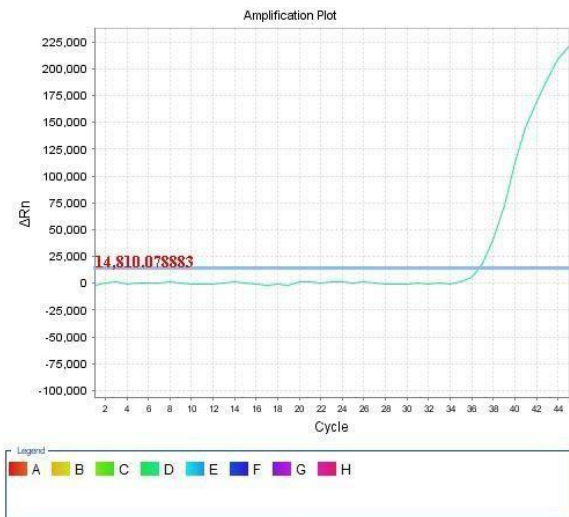


Figure 1. positive control



NEGATIVE CONTROL

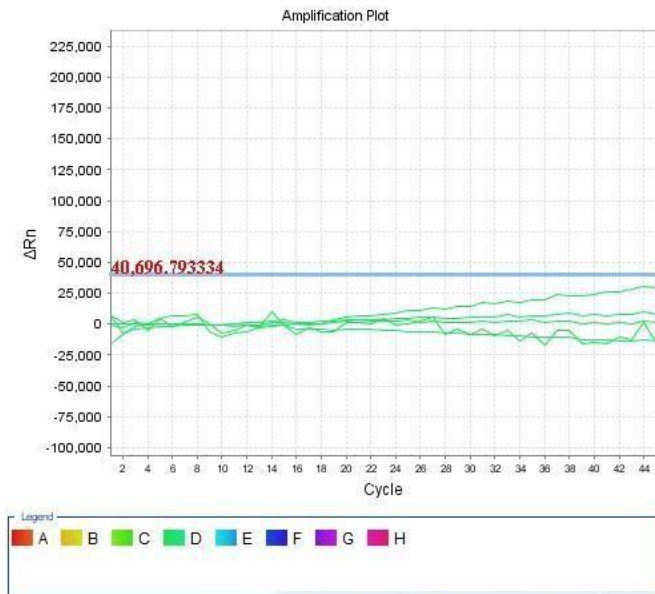
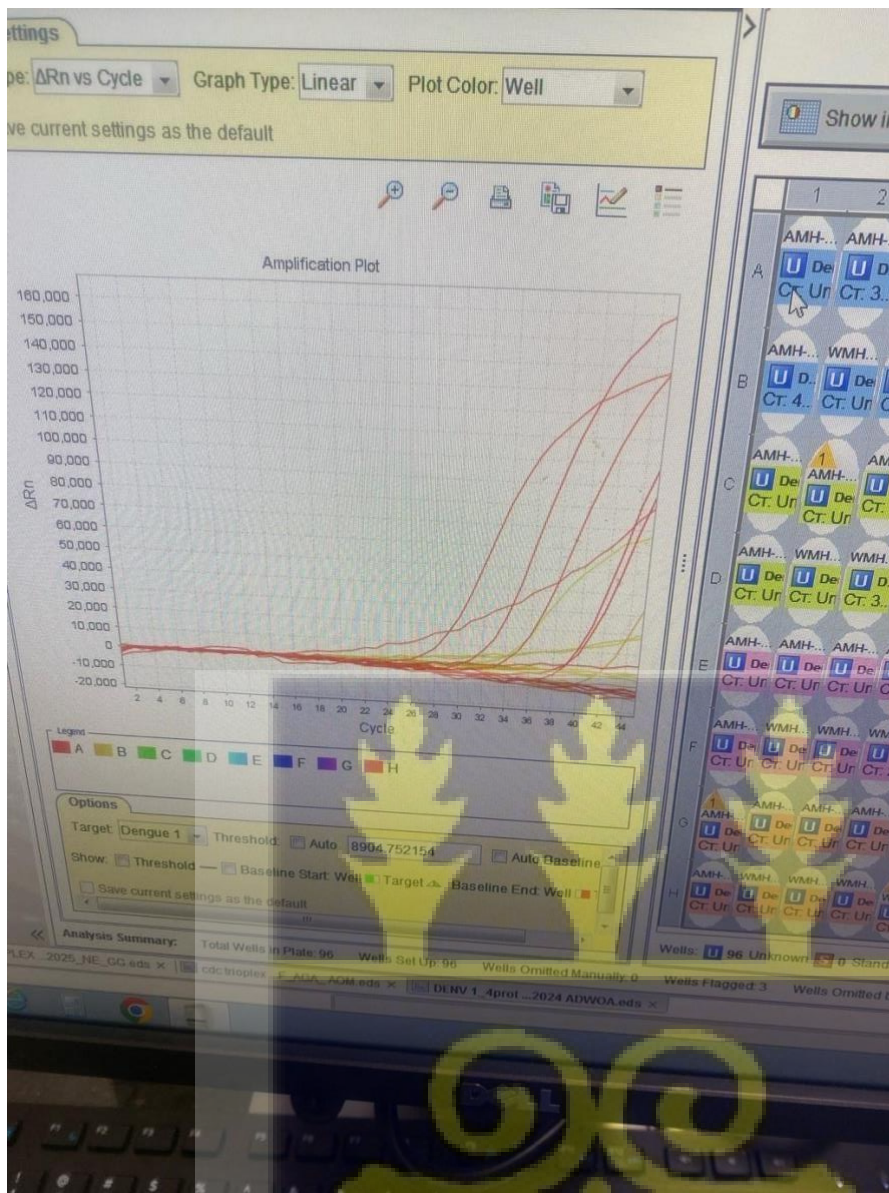


Figure 2. Negative Control



Real Time PCR For Dengue Serotyping.



Gel electrophoresis

A LABELLED GEL IMAGE OF DENGUE CONVENTIONAL PCR USING DUC/DUS PRIMER FOR MASTERMIX PREPARATION

DUC and DUS—expected size 511bp

For Positive control, DC-24-409 was used

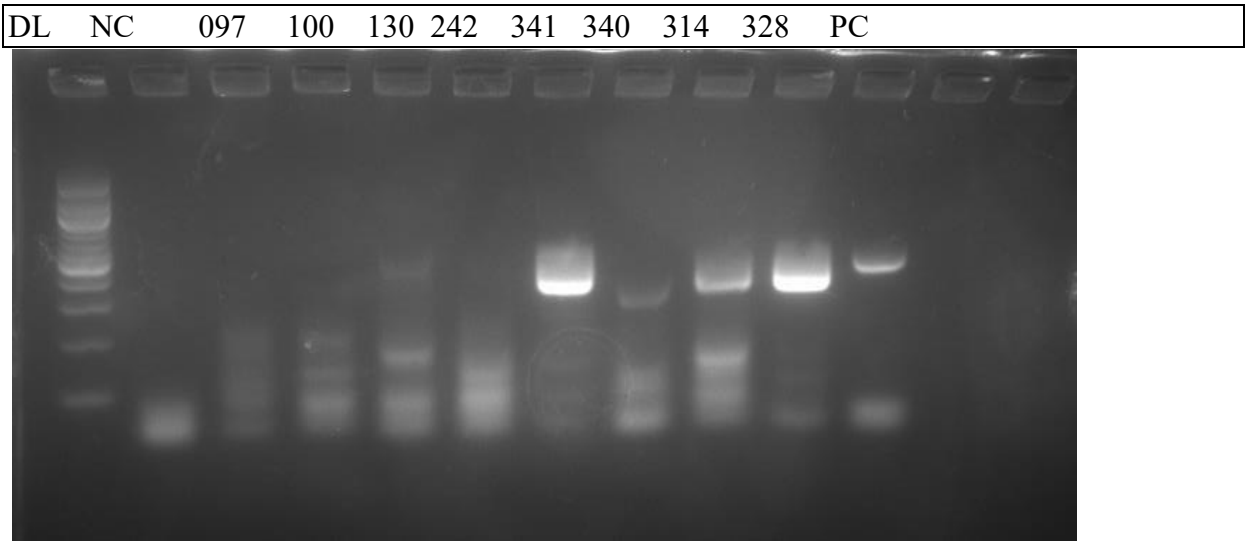


Figure 3: Results for Agarose gel confirming NS1 antigen

1.3 APPENDIX 4. QUESTIONNAIRES

Dear participant, I am Adwoa Twumwaah Gyapon (Student ID: 11006627) from the Department of Medical Microbiology, University of Ghana. Currently, I am pursuing a master's programme in MPhil Medical Microbiology. As a requirement for my degree, I am conducting a study on "Dengue viral Infection among febrile patients in selected Hospitals in Bono east Region in Ghana

As part of this study, you will be required to provide blood samples to be used for research purposes.

Therefore, I need your sincere cooperation to complete this questionnaire. I would appreciate if you could spend a few minutes of your time to complete my research. The data collected from this survey will be used strictly for academic purposes. Your participation is completely voluntary, and information will be kept strictly confidential. Thank you for participating in my research.

a. Demographic and geographical data

Id:

1. Sex ☐ M ☐ F

2. Age ____/____/____

☐ Unknown: Age: (year)

3. Level of education ☐ Primary

☐ Secondary

☐ High School

☐ Unschooled

4. Residence:

5. Do you know of anyone in your neighborhoods child has had dengue fever? ☐ No ☐ Yes

If yes, specify:

b. Clinical information

1. Date of admission (dd/mm/yyyy)/...../.....

2. Have you had any fever episodes in the last 3 months? ☐ No ☐ Yes

☐ No

3. Have you had any of the following symptoms in the last 3 months? ☐ Sudden onset of fever over 38.5°C

☐ Headaches

- ☐ Joint pain
- ☐ Muscle pain
- ☐ Lumbar pain
- ☐ No

Other specify:

4. Have you had any symptoms that could be caused by a disease other than dengue fever (cough, runny nose, sore throat, difficulty breathing, infected wound, etc.)? ☐ Yes

☐ No

If yes, specify:

5. Have you had any other signs? ☐ Pain behind the eyes

- ☐ Vomiting/ nausea
- ☐ Swollen glands
- ☐ Rash
- ☐ General fatigue
- ☐ Refused Meals
- ☐ Gastrointestinal disorder
- ☐ Bloody urine

If other, specify:

6. Were you away from home in the 15 days before the onset of fever (work, school, holiday, weekend)? ☐ Yes

☐ No

If yes, specify the place:

7. Where do you think you were bitten by a mosquito? ☐ At your home

If other place, specify: c.

Information on the sample

8. Was a blood sample taken from the case? ☐ Yes ☐ No

9. Blood sample ID (identical to patient ID)

10. Date of blood sampling/...../.....

11. Date sample sent to reference laboratory/...../.....

12. Did they take anything for treatment ☐ Yes ☐ No

If yes specify.....

13. Are there flower pot in your house. Yes ☐ No ☐

14. Have you travelled recently? if yes state ☐ where

15. Are there empty cans, old car tires or anything that would collect water? Yes ☐ No ☐

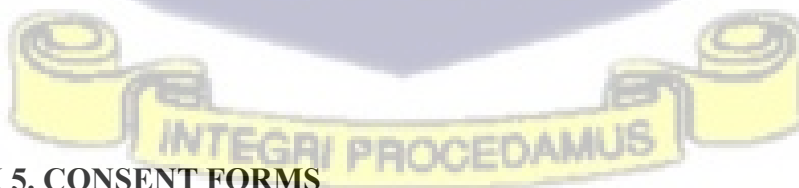
16. Do you store water in containers at home? Yes ☐ No ☐

17. Are the containers covered? ☐ Yes ☐ No

18. Do you have a gutter in front ☐ of your ☐ house? Yes ☐ No ☐

19. Is the gutter cleaned or dirty? Yes ☐ No ☐

20. Religion



1.4 APPENDIX 5. CONSENT FORMS

**Child Assent Form Project title: Dengue Viral Infection among febrile pediatrics
Populations in two selected hospitals in Bono East Region Researcher: Adwoa
Twumwaah Gyapon**

Address: Noguchi Memorial Institute for Medical Research. Telephone: 0541476796. Email:
adwoagyapon@gmail.com

Note: This form should be used only for children who are 1-17 years old Introduction

My name is and I am part of a team from Ghana

Infectious Disease Centre. I am undertaking a research study entitled “Dengue Viral Infection among febrile Pediatrics in two selected Hospitals in Bono East Region”. You are invited to participate in this research study to help investigate and gain a better understanding of the subject under study. if you have previously or currently been infected with dengue virus (germ). The duration of participation in the study will take one day.

General Information

If you choose to participate in this study, you will be asked about your health status and provide a small blood sample (approximately 5 mL or one teaspoon) for analysis. These samples will be tested to determine whether the dengue virus or other pathogens are responsible for fever. To ensure confidentiality, your samples will be assigned a coded identifier. Additionally, your body temperature, weight, and height will be measured, and your parents or guardians will be asked questions regarding socio-economic and demographic factors. Laboratory analyses will be conducted using available hospital equipment and specialized facilities at the Noguchi Memorial Institute for Medical Research, University of Ghana.

Some tests may require extended processing time; therefore, samples may be stored for future analysis and retesting, if necessary, after which any remaining specimens will be properly discarded. If you are requested to return for additional testing, including a second blood sample (5 mL) for antibody assessment, your transportation costs will be reimbursed.

Possible Risks and Discomforts

A blood sample will be obtained from a vein in your arm to test for the presence of the virus. Potential risks associated with blood collection are minimal but may include fainting, infection, mild discomfort, bruising, bleeding, or swelling at the puncture site. Proper skin disinfection will

be performed to minimize infection risk, and trained healthcare professionals will conduct the procedure. The volume of blood drawn is not significant enough to pose a health risk, though slight pain may be experienced when the needle is inserted. A physician will be available to manage any unexpected adverse reactions during the procedure.

Possible Benefits

Information from this study will tell us if dengue virus (germ) is circulating in locality and Ghana. This study aims to identify diseases other than malaria that contribute to acute febrile illness, improving diagnostic accuracy and treatment strategies. All participants will receive dengue virus testing at no cost.

Confidentiality

Confidentiality will be strictly maintained following Ghana Health Service standards, using nonidentifiable study codes. Participant names will not appear in publications, reports, or presentations. Personal records will be securely stored in locked files and protected computer systems, accessible only to key investigators. Individual laboratory results will be shared only with clinical personnel managing participant care. All paper records will be securely disposed of after five years.

Compensation

For those participating in the study at a healthcare facility, you will be given two cakes of soap and/or biscuit (children) to compensate for your time and participation.

For those participating in the study in the community, you will be given a cake of soap and a dewormer. Monetary compensation will not be provided for participation in the study.

Voluntary Participation and Right to Leave the Research

Enrollment in this study is completely voluntary, and individuals may withdraw at any point without facing any consequences. Additionally, participants have the right to decline answering any question that causes discomfort.

Your Rights as a Participant

Participants may withdraw consent and discontinue involvement at any time without consequences. Participation does not waive any legal rights or claims. For inquiries or concerns regarding the study or consent process, participants may contact the research team.

Persons to contact

If you have any inquiries regarding this study at any point, you may reach out for further information contact Dr. Mildred Adusei-Poku. on telephone number 0208821313 or write to her through this address: University of medical Microbiology. Red Building, Korlebu Medical Campus. P.O. Box 4236, Accra. For any inquiries regarding your rights as a participant, you may contact the Chairman of the Ghana Health Service Ethics Review Committee. Additionally, you can reach out to a representative of the committee at 02xxx for further information.

Voluntary Agreement

By signing or providing a thumbprint below, you confirm your understanding of the research study. If you choose not to participate, do not sign this assent form. A copy will be given to both you and your parents upon signing. The details of the study, including its benefits, risks, and procedures, have been explained to me, and I have had the opportunity to ask questions, which have been satisfactorily addressed. I voluntarily consent to participate in the study titled “Dengue infection in febrile pediatric Population in two selected Hospitals in Bono East Region in Ghana

Child's Name _____

Child's Signature/Thumbprint
same as subject's)

Date (must be the

☐ I agree that my specimen is store for future

If the participant is unable to read the form independently, a witness must provide a signature. The witness confirms that the participant has either read or been read the study information, demonstrated comprehension, and had the opportunity to ask questions, which were satisfactorily addressed. Additionally, the participant has been informed that involvement in the study is voluntary and that they may withdraw at any time without providing a reason.

Witness' Name _____

Witness' Signature/Thumbprint

Date (must be the same as subject's)

Signature of Investigator

I have provided the subject or their legal guardian with an explanation of the research, addressed all questions, and confirmed their understanding and voluntary consent to participate.

Thumbprint

Name of Investigator _____

Signature of Investigator

Date (must be the same as subject's)



9.2 APPENDIX 5 . PARTICIPANTS INFORMATION SHEETS

Title of Research Project: Dengue viral infection among febrile pediatrics Population in two selected Hospitals in Bono East Region, Ghana.

Principal Investigator: Miss Adwoa Twumwaah Gyapon.

Address; Noguchi Memoria Institute for Medical Research. P.O. Box LG 581, Legon.

Telephone: +233(0)541476796. **Email:** adwoagyapon@gmail.com.

Introduction

I, Gyapon Twumwaah Adwoa, MPhil Medical Microbiology student of the University of Ghana, am requesting your participation in the research study mentioned above. This study aims to provide information on its objectives, along with the potential risks and benefits, to enable you to make an informed decision regarding participation.

Purpose of the study:

Dengue fever is a mosquito-borne viral disease prevalent in tropical and subtropical regions, including Ghana. The mild form presents with high fever and symptoms resembling malaria, while severe dengue, or dengue hemorrhagic fever, can lead to significant bleeding, shock, and fatal outcomes. Millions of infections occur globally each year, with the disease being most common in specific endemic areas (Southeast Asia, the western Pacific islands, Latin America) including Africa. Ongoing research focuses on developing treatments and vaccines for dengue fever. Currently, in endemic regions, prevention relies on minimizing mosquito bites and implementing measures to control mosquito populations.

Many people experience no signs or symptoms of a dengue infection. When symptoms do occur, they may be mistaken for other illnesses such as malaria or flu and usually begin four to 10 days after you are bitten by an infected mosquito.

Results of this project may enable researchers to detect and understand whether dengue virus is circulating in the community and you have been infected with the virus now or in the past. The findings will help in the implementation of control and prevention measures in the future.

Possible Risks and Discomforts

Blood samples will be obtained from a vein in the arm for pathogen detection. While rare, potential risks include fainting, infection, minor pain, bruising, swelling, or bleeding at the puncture site. Proper skin disinfection and collection by trained personnel will minimize these risks. The blood volume drawn will not pose any health concerns. A medical doctor will be available to address any adverse reactions during the procedure.

Possible Benefits

You will not derive any direct benefit. However, information from this study will tell us if dengue virus (germ) is circulating in locality and the result will help in the implementation of control and preventive measures. These studies will provide valuable insights into non-malarial causes of acute

febrile illness, enhancing diagnostic and treatment approaches. Additionally, all participants will receive complimentary laboratory testing for the dengue virus.

Confidentiality

Participant confidentiality will be maintained by assigning unique identification codes to the data, ensuring anonymity of personal information. Only the principal investigator and the research supervisor will have restricted access to participant data. Confidentiality will be maintained in accordance with Ghana Health Service standards through the use of anonymized study identification codes. No individual names will be disclosed in any form of identification. reports, publications, meetings, or abstracts resulting from the study. Volunteer names and corresponding study numbers will be securely stored in locked physical files and encrypted digital records, accessible only to authorized investigators. Results from individual laboratory analysis will be shared with participants and healthcare providers involved in their care. All paper records will be securely disposed of after a retention period of five year.

Voluntary Participation/ Withdrawal

Participants may withdraw their consent and discontinue involvement at any time. They also have the right to decline any question and seek clarification on the procedure by contacting the principal Investigator.

Questions, concerns or complains

For any inquiries regarding this study, you may reach Dr. Mildred Adusei-Poku via telephone at 0208821313 or correspond with him through the following address. University of medical Microbiology. Red Building, Korlebu Medical Campus. P.O. Box 4236, Accra. For inquiries about your rights as a participant in this study, you may reach out to the chairperson of the Ghana Health Service Ethics Review Committee. Additionally, you can contact a representative of the committee at the Ghana Health Service via 02 for further details.



