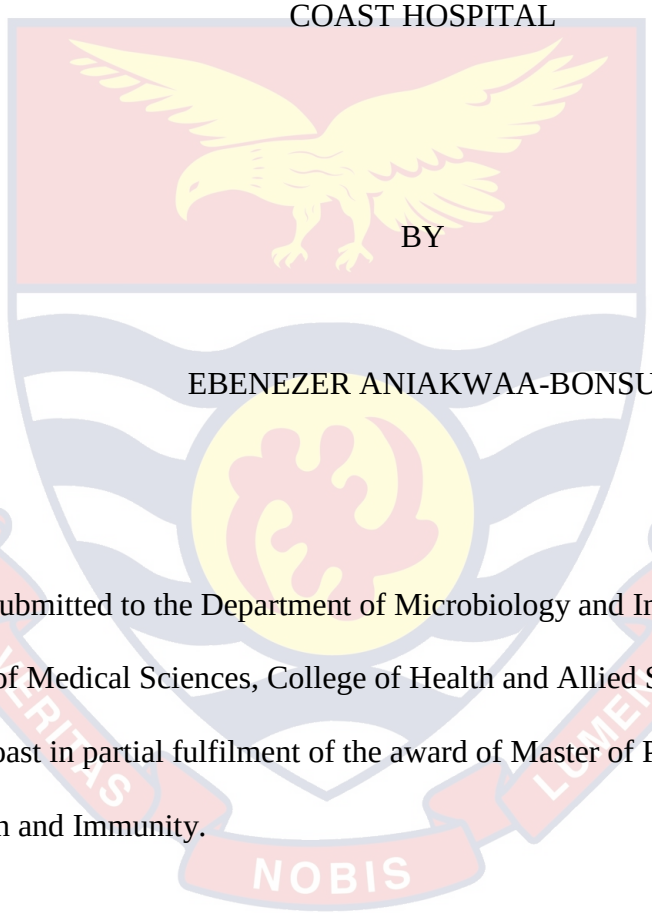
UNIVERSITY OF CAPE COAST

SEROPREVALENCE AND MOLECULAR DETECTION OF DENGUE VIRAL INFECTION AMONG ADULTS ATTENDING THE UNIVERSITY OF CAPE COAST HOSPITAL



BY

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Thesis submitted to the Department of Microbiology and Immunology of the School of Medical Sciences, College of Health and Allied Sciences, University of Cape Coast in partial fulfilment of the award of Master of Philosophy degree in Infection and Immunity.

OCTOBER 2020

DECLARATION

Candidate's Declaration

I hereby declare that except for reference to other people's work which have been duly acknowledged, this piece of work is my own composition and neither in whole nor in part has this work been presented for the award of a degree in this university or elsewhere.

Candidate's Signature…… Date…….

Ebenezer Aniakwaa-Bonsu

Supervisor's Declaration

I hereby declare that the preparation and presentation of the thesis was supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Supervisor's Signature……. DATE…….

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ABSTRACT

Most febrile illnesses in Ghana are often misdiagnosed and presumptively treated as malaria. This study sought to investigate the seroprevalence, detection the viral RNA, determine geographical location of participants with circulating antibodies and virus and finally ascertain the diagnostic accuracy of an RDT kit. A hospital-based cross-sectional study was conducted among adults (≥18) with at least three malaria-like symptoms attending the University of Cape Coast Hospital. From each participant, 3ml of blood was drawn and serum was tested for IgG and IgM using RDT and ELISA. Seropositive samples were selected for PCR testing. Statistical analysis was performed using STATA (v.14) software. A total of 270 participants were enrolled in the study. The median age was 31 with 23 and 43 as their interquartile ranges. Seroprevalence of IgG and IgM by ELISA was 12.6% and 2.2%. Overall seroprevalence was 12.96%. Females recorded a high seropositivity rate (7.4%) than males (5.2%) in terms of past exposure (IgG). On current exposure (IgM), females still recorded a high seropositivity rate (1.5%) than males (0.7%). Seroprevalence of individuals recent secondary infection (IgG+IgM+) was 1.85%. Those with primary and recent infection (IgM+IgG-) were 0.37% whiles those with past and probable secondary infection (IgG+IgM-) were 10.7%. Elmina neighbourhood was shown to have the highest seropositivity rate values for both anti-dengue IgG (3.3%) and IgM (1.85%). Kappa value for RDT was 0.37 and 0.0001. The Ministry of Health should create awareness and enhanced dengue surveillance.

KEYWORDS

Enzyme-Linked Immunosorbent Assay

Dengue virus

Immunoglobulin M and G

Rapid Diagnostic kit

Reverse Transcription-Polymerase Chain Reaction

Seroprevalence

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DEDICATION

I dedicate this work to my parents, wife, siblings and my uncle (Prof. Anthony Kwame Nyame).

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LIST OF ACRONYMS

ADE	Antibody-dependent enhancement
CprM	Core Pre-Membrane Region
DF	Dengue Fever
DHF	Dengue Haemorrhagic Fever
ELISA	Enzyme-Linked Immunosorbent Assay
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IVM	Integrated Vector Management
NS1	Non-Structural protein 1
OD	Optical Density
RNA	Ribonucleic acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
WHO	World Health Organisation

CHAPTER ONE

BACKGROUND

Introduction

This chapter provides background to the study with reference to relevant literature. Underreported cases of dengue, which is underpinned by lack of national surveillance, misdiagnosis, misclassification of cases, the use of unvalidated rapid diagnostic test among others have become a great public health concern. Studies of this nature have therefore become important in helping to create alert and awareness so that endemic countries like Ghana will heighten surveillance and outbreak preparedness in terms of human resource training and logistics, including diagnostics. In the light of this, the country will not be overwhelmed when significant number of haemorrhagic fever cases starts coming in. It also highlights chronologically on the problem statement, aim and specific objectives of the study, justification, significance, and the limitation of the study.

Background Information

Dengue virus infection is an evolving health threat that is now assuming considerable global public health concern. Since 2012, it has been shown to be the most serious arboviral disease transmitted by mosquito globally (WHO, 2012) with greater morbidity and economic impact (Gubler, 2012). Data from the World Health Organization suggested that an estimated 2.5 billion people dwell in endemic areas (WHO, 2009a) with over 50 million dengue infection occurring

yearly and with an estimated 19000 dengue-related deaths which were recorded in 2002 (WHO, 2006). Generally, the infection has been reported in the tropics and sub-tropics of the globe (WHO, 2006). In non-endemic areas, the bulk of notified cases of the infection is mostly spread by international travelers that have visited dengue-endemic areas (WHO, 2006).

Dengue has also been recorded in Africa but most often without laboratory confirmation (WHO, 2009a). Seroprevalence (Brady et al., 2012) have revealed dengue exposure in sub-Saharan Africa. West Africa is a probable region for the viral transmission due to the presence of the transmitting vector, urbanization, poor sanitary measures and inadequate diagnostic tool (Stoler et al., 2014). DEN-1, -2 and -3 were first isolated in West Africa in the 1960s from human samples in Nigeria (Guy & Almond, 2008). Other epidemics were reported in Burkina Faso and Senegal in 1982 and 1999 respectively (Parks et al., 2004). Cases of DEN-1 and 2 outbreak were recorded in Cote d'Ivoire in 2006 and 2008 respectively (WHO, 2009a), and an outbreak of DEN-2 occurred in Burkina Faso in 2016 whiles DEN-3 and 4 were recorded in East Africa in 2001 (Amoako et al., 2018).

In Ghana, few studies have been conducted with a seroprevalence of 43.6% in blood donors at a Teaching hospital in the Ashanti Region (Narkwa et al., 2016). Humphery and colleagues (2018) detected dengue among 150 patients with suspected Ebola virus disease. In this study, 15% tested both positive for IgG and IgM, 4% for non-structural protein 1 (NS1) whiles 4 individuals tested positive for RT-qPCR.

The awareness of the clinical and economic burden of a disease is important as far as the development of efficient public health policies and the allocation of healthcare resources are concerned. Between 2000-2007, the financial burden of dengue was approximately US$ 2.1 billion in the Americas alone and the global disability-adjusted life years (DALYs) due to the disease was 700000 in 2009 (Ayukekbong et al., 2017). The Global Burden of Disease study that was done in 2013 revealed that dengue was responsible for global estimation of 1.4 million disability-adjusted life years (DALYs). In the study, they reported a global burden of US$ 8.9 billion (Hung et al., 2018). In another study, dengue was accountable for 1.14 million DALYs in 2013 considering lethal and non-lethal outcomes (Stanaway et al., 2016). In a work that was done to assess the global burden of dengue, an average dengue death of 9221 was reported per year between 1990-2013. This generated a total of 576 900 years of life lost to premature death attributable to dengue. The disability years lived accounted for moderate and severe dengue and post-dengue were 566 000 years in 2013 (Stanaway et al., 2016).

Dengue virus is a positive-sense RNA flavivirus that is spread by an insect vector called *Aedes aegypti*. Antigenically four different serotypes exist. They are identified as DEN-1, DEN-2, DEN-3, and DEN-4 (Holmes & Twiddy, 2003). Infection with any of the above serotypes may show no noticeable signs or may produce a range of clinical signs such as undifferentiated fever (UF), Dengue fever (DF), Dengue Hemorrhagic Fever (DHF), and Dengue Shock Syndrome (DSS) (WHO, 2009a). Lifelong immunity is achieved to a serotype when an

infection of that serotype is established (Changal et al., 2016). Normally, dengue fever is a self-limiting disease manifested by fever, headache, arthralgia, retro-ocular pain, and myalgia. The symptoms and signs may be very similar to malaria and other fever causing viral infections (WHO, 2012).

The signs of dengue fever typically mimic febrile illnesses such as malaria, hence, it is presumptively treated as malaria in most endemic countries (Ayukekbong et al., 2017). In a study of 605 feverish children in Ghana, only 11% tested positive for malaria by microscopy after 80% had been diagnosed with malaria (Stoler et al., 2015). This data suggest that the etiology of febrile illness goes beyond Plasmodium infections. There is, therefore, the need to do routine and differential diagnosis of dengue so there can be a laboratory evidence-based treatment. This will aid in the reduction of indiscriminate use of antimalarial and antibiotics to reduce resistance in circulation.

Dealing with the viral transmission is hinged on controlling the disease vectors or interrupting the contact between humans and the vector. The World Health Organisation (WHO) has therefore promoted a strategic approach known as the Integrated Vector Management (IVM) which seeks to improve efficacy, cost-effectiveness, ecological soundness, and sustainability. This approach involves the use of integrated control methods which include environmental management, biological and chemical control which has been shown to reduce vector

transmission significantly WHO, 2012). To augment and improve the efficacy of the traditional IVM control approach, there has been the introduction of biotechnological interventions such as paratransgenesis which is a tool used to

genetically engineer a symbiont mosquito, to deliver antipathogen effector molecules, thereby decreasing the vector competence to the pathogen. Another biotechnology intervention tool is the sterile insect technique (SIT) where sterile male mates with females to produce no off springs; in this way, reducing the next generation population.

One essential tool in addition to the above methods in dealing with mortality and morbidity of dengue viral infection is health education. Health education is essential in ensuring that the general population understands the mechanisms, transmission, and the behaviours that need to be addressed to prevent disease transmission, reduce severity, and avoid fatalities (Hadinegoro, 2012). Health surveillance which is prerequisite tool for dealing with disease such as dengue are; timely detection of epidemics for early intervention, measurement of disease burden and its clinical, economic and social impact in affected areas, monitoring the trends in its distribution over time, its geographical location, and evaluating the effectiveness of its prevention and control programme (WHO, 2009b). This research sought to address some of these issues by determining the seroprevalence and molecular detection of dengue, its circulating antibodies to predict the state of the infection in the population and the geographical distribution of the virus.

Problem Statement

The fever phase of dengue viral infection presents with symptoms like other febrile illnesses such as malaria. This is often characterized by arthralgia, myalgia, retro-orbital pain, fever, nausea, vomiting, back pain (Lim et al., 2018a). Most febrile illnesses in Ghana are often misdiagnosed and presumptively treated

as malaria or pyrexia (Stoler *et al.*, 2018). The consequences of this is the development of drug resistance to antimalaria drugs and antibiotics.

According to the WHO, surveillance data of the disease in the African is poor and even outbreak reports are underreported and misclassified. Nonetheless, there is evidence suggesting an increase in the size and frequency of the infection (WHO, 2009a). Though surveillance data in Africa is poor, the four serotypes responsible for epidemic dengue fever have shown to increase dramatically since 1980 (WHO, 2009a).

Dengue outbreaks have been reported in two countries that share borders with Ghana (WHO, 2004, 2009a) and this may be due to environmental conditions that favour the breeding of the viral-borne vector *Aedes aegypti*. Amoako and colleagues (2018) reported that, a DEN-2 virus which was isolated from a child with suspected malaria in Accra had a close relation with DEN-2 which was implicated in the outbreak in Burkina Faso. As Ghana shares boarder and similar conditions which are risk factors for viral transmission, it is a potential hotspot zone for transmission of the virus.

Although there are no official records on the dengue virus outbreak in Ghana, few studies done have confirmed the presence of dengue infection and its serotypes (Narkwa et al., 2016). The paucity of research and surveillance data in Ghana suggests that the sporadic cases of dengue viral infections (DENV) have been ignored indicating latent endemicity of the dengue infection in the general population. This implies that there is potential risk for dengue outbreak as several serotypes may be circulating among the human population putting them at risk of

antibody-dependent enhancement (ADE) dengue haemorrhagic fever (DHF) and dengue syndrome shock (DSS) if there is secondary infection with a heterologous serotype.

Significance of the Study

The dengue viral infection has been reported to be in Ghana at least since 2013 (Narkwa et al., 2016). The latent endemicity and circulation of the variant serotypes predispose individuals with primary infection to dengue haemorrhagic fever. Therefore, studies conducted on the prevalence, coupled with close monitoring of cases and case detection in the health facilities is vital in the understanding of the burden of the disease. It is anticipated that the findings of this research alongside other studies done earlier will create an awareness, enhance national surveillance and preparedness so that the nation will not be overwhelmed when more cases of haemorrhagic fever begins to show up in significant numbers. It will also help medical practitioners to consider severe dengue as a possible clinical case when dealing with febrile patients, so that they can be given the required and proper care to avoid severe and fatal complications.

Objective

The aim of the study is to determine the seroprevalence and molecularly detect dengue virus in adults attending the University of Cape Coast hospital.

Specific Objectives

1. To determine the seroprevalence of anti-dengue IgG and IgM antibodies among study participants using the RDT and ELISA method.

2. To evaluate the performance characteristics of a rapid diagnostic test (RDT) using ELISA as a gold standard.
3. To isolate the viral nucleic acid among the study participants using the PCR method
4. To determine the geographical location of the participants with circulating antibodies and virus.

Delimitation of Study

Participants recruited for this study were patients who were attending the University of Cape Coast Hospital, in Cape Coast at age 18 years and above showing at least three malaria-like symptoms.

Limitations of study

This study had a limitation. The flavivirus group of viruses have a similar genome hence cross-react with each other. There was therefore the need to confirm samples that tested positive for IgG and IgM with the plaque neutralization reduction test (PRNT); this was not done due to its unavailability

Organisation of Study

The study is organized into five main chapters. Chapter one comprises information on study background, problem of study, its significance, objective and specific objectives, delimitation, limitation and summary. In chapter two, the study reviewed and discussed relevant literature of the study which includes, geographical distribution of dengue virus, its case detection and management, major gaps in the disease surveillance and the global effort to reduce disease

morbidity and mortality. Chapter three highlighted on research design, study area, population, laboratory procedure, data analysis and ethical issues. Chapter four focused on results and discussion of results using relevant literature. The last chapter concluded on the major findings of the study and made necessary recommendations

Chapter Summary

This chapter emphatically stated the aim and objective of this study and with reference to relevant literature, highlighted on the public health implications of dengue globally and locally. It detailed on the economic impact and clinical burden burdens. Again, it revealed the classical symptoms of the disease and the mechanism of these symptoms. It is also touched on the transmission and its transmitting vector and disease control. Again, it discussed the problem associated with its diagnosis in health facilities and establishes why it should be considered as a possible case when clinicians are dealing with febrile conditions. Finally, it mentioned the limitation of the study.

CHAPTER 2

LITERATURE REVIEW

Introduction

This chapter discusses the related books and theories relevant to the research topic. The concept of the research are used in reviewing the relevant literature related to the research topic and these include; global and geographical distribution of viral serotypes, surveillance, case detection and management, outbreak preparedness, major gaps in the knowledge of the true burden of the disease and the World Health Organization global strategy for prevention and control.

Global Distribution

Severe dengue epidemic had been experienced by only nine countries before 1970 (WHO,2012). The infection is widespread in over hundred tropical and subtropical regions across the globe with the greatest burden of the disease within the South Eastern Asia Region (Guo et al., 2017). Risk factors (i.e. rainfall, warm temperature, increasing urbanization, poor sanitation, presence of vector) are contributors to the transmission of dengue throughout the tropics and subtropics of the globe. The WHO reveals that cases of dengue are not adequately reported (WHO, 2012). Studies have shown an annual infection of 390 million with 96 million clinical manifestations (Bhatt et al., 2013) with a 20% case fatality rate (Guo et al., 2017). Two-fifth of the world's population according to some studies

are at risk of the infection (Screaton et al., 2015). Since 1980, there has been an increase in dengue outbreaks in Asia, South America, and the Caribbean. A total of 291,964 dengue associated outbreak was reported by the end of 2016: largely from China, Singapore, and Malaysia. Other cases of dengue were reported in the Western Pacific, America, South East Asia, Eastern Mediterranean, European and the African regions (Guo et al., 2017).

Threat of outbreak now exist in Europe (ECDC, 2019). In 2010, 1143 cases were detected in European countries such as France and Croatia and contributed to most hospitalization among travelers from the tropics and sub-tropics (Schaffner & Mathis, 2014). In 2012, there was a reported outbreak in the Madeira Island in Portugal (ECDC, 2019) and this signals the introduction of a high-risk competent vector in Europe. From 2010, local cases of dengue are reported annually in Europe (Schaffner & Mathis, 2014).

Studies done in Africa have reported dengue cases in thirty-four countries (Were, 2012). Despite these reports in Africa, there is no proper surveillance system to monitor the disease (Beatty et al., 2010); the burden of dengue therefore remains unknown in Africa (Lim et al., 2018b). There was a case report of dengue between 1964 and 1968 in Nigeria (Messina et al., 2014). Outbreak owing to DEN-2 was the first outbreak reported in Burkina Faso (Lim et al., 2018b). A study conducted in 2006 in Burkina Faso showed a seroprevalence of anti-dengue antibodies to be 62.8% (Xue et al., 2017). In another study conducted among febrile patients, 8.7% were shown to be positive by rapid test while 35% were shown to be positive RT-PCR (Ridde et al., 2016). Seroprevalence in Gabon has

been shown to be between 5-20% while three serotypes have been reported to cause DHF cases (Melanie Caron et al., 2013; Mélanie Caron et al., 2012). Studies conducted among toddlers in Gabon showed a seroprevalence of 12.3% in a semi-rural setting (Lim et al., 2018a). In Kenya, a household survey study in the Mombasa county showed that 13% of individuals from 701 households were DEN-2 anti-dengue antibodies (Murray et al., 2013)

Global Distribution of Dengue Serotype

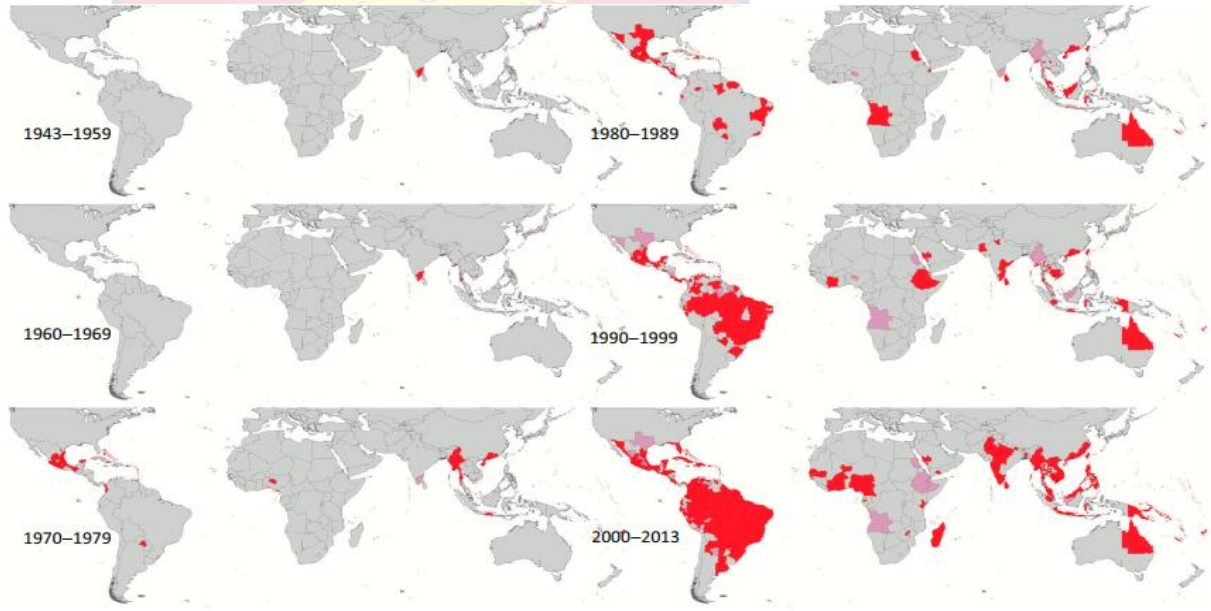

Fig. 1. Global distribtion of DEN-1 serotype (Messina et al., 2014)

Distribution of Serotype 1

The above figure shows the global distribution of dengue serotype 1 for a period of time. Japan and French Polynesia reported first cases of DEN-1 in 1944 whiles it was reported in Hawaii in 1945 (Messina *et al*., 2014). Sudan was the first country in Africa to report a case of DEN-1 in 1948 (WHO, 2006). Cases of

DEN-1 were reported in Saudi Arabia in the mid-1990s, mid-2000 and late 2000 (Messina *et al*, 2014). In the Caribbean, cases of DEN-1 were not reported until 1977 when countries like Barbados, Cuba, Grenada, Praguay and Puerto Rico recorded their first cases with serotype 1. Other countries in this region, which reported cases of DEN-1 in the 1980 includes Guiana, Costa Rica, Venezuela, Peru, and Columbia (WHO, 2012).

Distribution of Serotype 2

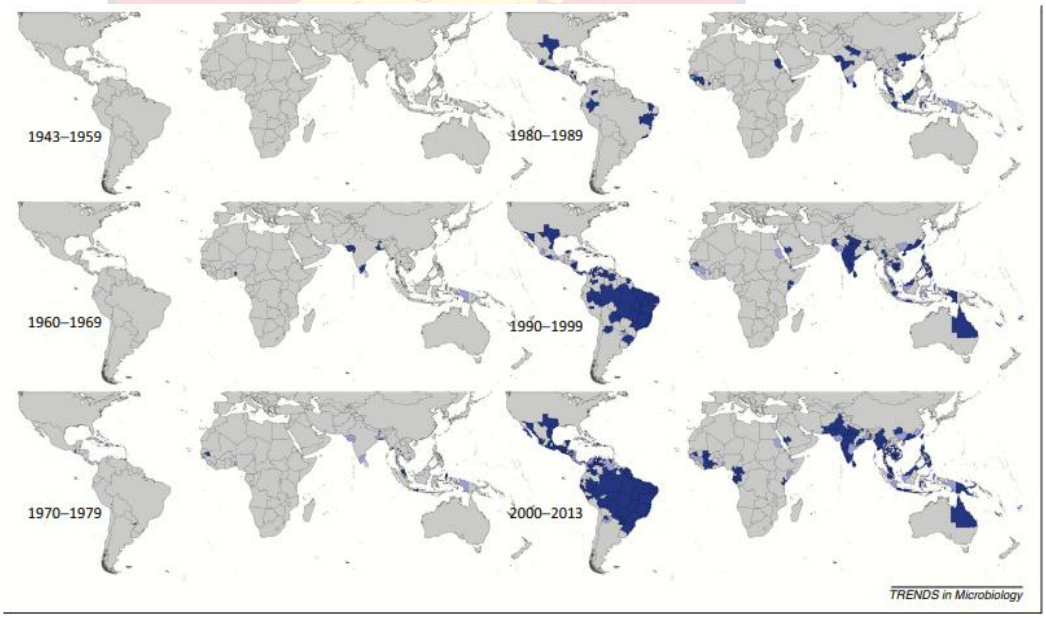

Fig. 2. Global distribtion of DEN-2 serotype (Messina et al., 2014)

The first cases of DEN-2 were reported in Indonesia and Papua New Guinea in 1944 and the cases were reported in the Philippines in 1944 (Messina *et al.*, 2014). China, Singapore, Sri-Lanka have all reported cases of DEN-2 (Stanaway et al., 2016). In Africa, Nigeria was the first country in 1964 and 1968 to report cases if DEN-2 but has since not been reported (Ayukekbong et al., 2017).

Sporadic cases have however been reported in Africa with the most recent in Burkina Faso in 2016. In America, there has been DEN-2 reports in Trinidad and Tobago, Puerto Rico, and Brazil since 1984 with increasing cases of dengue haemorrhagic fevers (Messina *et al.*, 2014) which was due to imported cases of the more virulent Asian strain one of DEN-2 ((Rico-Hesse et al., 1997).

Distribution of Serotype 3

First cases of DEN-3 were reported in the Philippines and Thailand in 1953 and was reported in every region of Asia in 1962 (WHO, 2006). Throughout Asia, Thailand was known to notably record cases of DEN-3 every year between 1973 and 2010 with the greatest burden between 1999 and 2000. Malaysia Indonesia, Sri-Lanka, China, Vietnam, Cambodia, and Singapore recorded cases of DEN-3 since 1980 and was consistent to the middle of 1990 (Messina, 2014). Cases of DEN-3 in America were reported in Puerto Rico in 1963, which continued to 1978 and then in 1994, which continued to 2008 and this was as a result of the introduction of a new genotype of DEN-3 from Asia (Messer et al., 2003). Not until the late 1980s and the late 2000s most of countries in the Americas were not reporting cases of DEN-3 (Messina *et al.*, 2014). Data on DEN-3 is very scanty and unreliable; Mozambique reported its first case of DEN-3 between 1984-1985 (WHO, 2012). In the Middle East, Saudi Arabia reported the highest number of DEN-3 cases between 1994 and 2009 (WHO, 2006).

Distribution of Serotype 4

The first reported case of DEN-4 was in 1953 in the Philippines and Thailand with annual autochthonous cases (Iguchi et al., 2018). Frequent cases of DEN-4 have also been reported in Asian countries like Sri-Lanka, French Polynesia, Indonesia, India, and Myanmar. It has also been reported in the Americas in countries like Brazil, Cuba, Dominica, Puerto Rico, Nicaragua, Columbia, Venezuela and the US Virgin Islands (Messina *et al.*, 2014).

Reducing morbidity and mortality

Technical strategies and enabling factors are essential for reversing the increasing trend in the number of dengue cases. Mortality can be reduced by the implementation of early case detection, managing severe cases with the required treatment, orienting health facilities to cope with dengue outbreaks and giving adequate health training to health personnel (WHO, 2012). Morbidity can also be reduced by improving case detection (Stoler et al., 2015), enhanced entomological and epidemiological survey through the promotion of integrated vector management, efficient urban management, and creation of awareness to achieve behavioral outcomes in preventive programmes.

Case management and diagnosis

Early clinical and laboratory diagnosis of dengue need to be enhanced through timely implementation of required clinical management (Blacksell et al., 2006). There is therefore the need to provide accurate diagnosis, assessment, and

confirmation because this is particularly important in the reduction of mortality cases.

The infection has a wide spectrum of clinical manifestation ranging from mild to severe. Prior to the three phases of the infection; febrile, critical and recovery, the illness most often begins abruptly after the incubation period (WHO, 2009a). For an infection such as this, a rational case management yielding good clinical outcome is underpinned by early detection and the understanding of the clinical challenges during the different phases of the disease especially in dengue haemorrhagic fever. A well-managed response from frontline health workers will reduce unnecessary hospital admission and also reduce cases of severe dengue. Training for all health workers involved in the clinical management of dengue is therefore very crucial. Health Agencies should also create awareness, particularly in endemic areas so individuals can be aware of the early signs and symptoms of the disease so to improve on their health seeking behaviours.

Diagnosis of dengue with only signs and symptoms can be confusing with other diseases such as malaria, leptospirosis, typhoid fever, and other infections caused other arboviruses and this is why differential diagnosis is very critical ((Muller et al., 2017a). Diagnosis may include the detection of viral nucleic acid, antibodies, and viral antigens. After disease onset, the virus and antigen can be detected in blood cells, serum, and plasma for an average of five days (WHO, 2012). Non-structural protein 1 (NS1) antigen, virus isolation by cell culture and nucleic acid detection is very useful in the acute phase of the infection diagnosis. Serological assays used in the detection anti-dengue IgG and IgM, are very useful

after the acute phase of the infection (WHO, 2012). A primary dengue infection stimulates a stronger IgM response but with a secondary dengue infection stimulates a stronger IgG response but a weaker IgM response (WHO, 2009a).

Surveillance and outbreak preparedness of dengue

According to the World Health Organisation (2009), epidemiological surveillance is the ongoing systematic collection, recording, analysis, interpretation, and dissemination of data reflecting the current health status of a community or population so that action may be taken to prevent or control a disease. This is critical in the prevention and control of dengue because it provides critical information required in risk assessment, epidemiologic response, and evaluation highlights on transmission risk.

The detection and forecasting of epidemic activity have been the main target of dengue surveillance and this includes human cases surveillance, laboratory-based surveillance, vector-based surveillance, and environmental risk factors for dengue (WHO, 2012). Augmenting effective surveillance includes financial support, human resource, training and necessary tools required for the early detection and response ((Harrington et al., 2013).

The prevalence and close monitoring of dengue over a time period will lay a baseline value of the rate of the disease and this can aid in the triggering of an alert, further investigation, intervention, and preventive measure appropriately. Early warning via early surveillance have helped in times past in epidemics in enabling health services to deploy health personnel and allocate the necessary

resources more effectively, engage in better community control programmes in reducing the spread of the disease (WHO, 2006).

Integrated surveillance

Dengue surveillance should be incorporated into the national health information system of countries within the endemic regions with set of indicators (Harrington et al., 2013). This integrated effort is crucial in the formulation of health policy-making, programme action and research. According to the WHO, some countries have routine and enhance health information coupled with epidemic disease monitoring system and it has aided them in them in greater efficiency and decision making at national, regional, provincial, and local levels. It is therefore imperative that a coherent effort be made towards integrated surveillance so to obtain a critical data of the global and national burden of the disease in order to assess progress in the reduction of mortality and morbidity goals. Most often, the data generated by national surveillance vary and in some endemic regions such as Africa, there is almost no data on incidence (WHO, 2012).

Outbreak preparedness

The overall strategy of surveillance includes outbreak preparedness; it forms an important operational and technical element. Its effectiveness is underpinned by a well-developed contingency plan, broadly disseminated, thoroughly understood and pre-tested before an epidemic (Peck, 2011; WHO, 2004). In preparing for dengue outbreaks, the plan should include well organized

administration and health services, logistic capabilities to deal with patients' inflow, medical supplies, adequate facilities and robust vector control efforts and communication with mass media. Again, aims, scope and objectives should be clearly stated in the plan. Rigau-Perez and Clark in 2005 highlighted on ten (10) priority areas for planning emergency response for dengue. These include: an establishment of a multisectoral dengue action committee, formalization of an emergency action plan , enhancement of disease surveillance, performing diagnostic laboratory test, enhancing vector surveillance and control, protecting special populations and reducing the impact of environmental determinants, ensuring appropriate patient care, engaging the communities and relevant profession al groups about dengue control as well as their population in dengue prevention and control, investigation of epidemic and finally, managing the mass media.

In countries with no record of circulating virus but with dengue vector present, management plans should focus on strategies to reduce the risk of transmission. These strategies include investigation of clinical suspected cases via laboratory confirmation, determine whether cases are imported or local-acquired and regularly monitor vectors mostly in the region of a suspected case. The focus of preparedness in countries where there is the risk of introducing dengue vectors should be on entomological surveillance, education of healthcare providers and the community about the risk of dengue (WHO, 2012).

Challenges in the knowledge of disease burden

The global burden estimation of dengue is uncertain. Under-reported cases contribute to most of the barriers in obtaining accurate estimation (WHO, 2012). Challenges which underpins under-reported cases include lack of uniform application of WHO case definition, limited capabilities and standards of dengue laboratories, limited accuracy of rapid diagnostic tests, misdiagnosis, lack of uniform criteria to report cases of dengue to WHO, limited role of surveillance and reporting system, underreporting of non-fatal and fatal dengue, misclassification of reporting dengue and misclassification in the reporting of dengue (WHO, 2009a).

Lack of uniform application of WHO case definition

Clinicians and researchers have reported difficulty in the application of WHO publishes guidelines for diagnosis, classification and management of dengue (Toledo Romani et al., 2007). Due to this complexity, some countries have instituted their own case definitions categories relevant to their country for case detections and management. (WHO, 2007).

Limited capabilities and standards of dengue laboratories

The accuracy of commercial assays used in the detection of anti-dengue IgG and IgM may vary most often with the situation in which they are used. Detection of virus using cell culture, or the polymerase chain reaction technique may require a more sophisticated research laboratory to perform quality test. Technical expertise and infrastructure needs to be enhanced in highly endemic regions

(Harrington et al., 2013). The WHO assessment of laboratories in the South Eastern Asia, Western Pacific and the Americas showed that although most of the laboratories had a high diagnostic accuracy in detecting IgG and IgM dengue, only 63% out of the 83 laboratories within these regions participated in quality control. Some challenges that were faced by participating laboratories included unavailability of antigens, low sensitivity of IgM antibody and lack of quality control information about dengue viral isolation.

In the Northern America and Europe where most cases are imported, they may overlook the disease owing to the fact that laboratory testing may not be available (Raafat et al., 2019). For instance, in the case of quality assurance evaluation in 18 European laboratories, samples that were tested for the detection of anti-dengue IgG and IgM concurrently reported 71% of IgG positivity and 58% IgM positivity. In the light of this, there is therefore the need to address worldwide laboratory capabilities and quality control (WHO, 2012).

Limited accuracy of rapid tests

In a comparative analysis of eight rapid diagnostic assays for dengue antibodies, their performance characteristics were evaluated . The analysis showed that all the eight RDTs had varying sensitivities (6%-77% and specificities (66%-100%) suggesting limited accuracy (Blacksell et al., 2006). Several studies (Agarwal et al., 2018; Raafat et al., 2019) have highlighted that most often, the sensitivity values as claimed by the manufacturers are not accurately so and therefore recommended proper validation for RDT kits. The

World Health Organisation (2012) has also express worry on the cost of RDT kits in developing countries and unvalidated kits sold on the market.

Misdiagnosis

Though WHO has given guidelines on diagnosing dengue, it is often misdiagnosed due to its unspecific symptoms which confuse clinicians with other infections like leptospirosis, chikungunya, malaria, typhoid, enterococcus and other viral haemorrhagic fevers. In a study by Stoler and colleagues (2018), 80% of febrile children who were diagnosed with malaria and presumptively treated as malaria did not actually have plasmodium infection upon further laboratory assessment. A six (6) month study in Texas showed that, among patients who were diagnosed with dengue, only 50% of them were correctly diagnosed (Cechinel et al., 2016). Again between 1995 and 1997 in Barbados, most people who were diagnosed with leptospirosis actually had dengue while those misdiagnosed with the later actually had leptospirosis (WHO, 2007). In the Bangladesh outbreak, it was shown that 18% of dengue suspected cases were actually leptospirosis upon further laboratory investigation (LaRocque et al., 2005).

According to WHO guidelines for malaria, febrile children with no alternative explanation who are aged 2 months to 5 years should be treated for ,malaria. This directive can contribute to the misdiagnosis of dengue especially in areas with low transmission risk of malaria where malaria diagnosis id not routinely done. Dengue is regarded as one of the neglected tropical diseases and in most endemic areas like Africa, it is not part of the usual routinely tested cases in clinical

laboratories and most often misdiagnosed as pyrexia or malaria (Stoler *et al.*, 2018). In countries with adequate laboratory facilities and routinely tested as a clinical case, there is the issue of cross-reactivity between anti-dengue antibodies and other flaviviruses like the Japanese encephalitis, Yellow fever, West Nile, St. Louis encephalitis).

Lack of uniform criteria to report cases of dengue to WHO

In the Americas, cases of dengue are reported to WHO by severity; dengue fever and dengue haemorrhagic fever. Meanwhile in the South Eastern Asia Region and the Western Pacific cases are reported without stratification of severity. Again, while some nations report on cases of DHF and dengue shock syndrome, others also report all clinical and confirmed suspected cases of dengue irrespective of their severity (WHO, 2020). This lack of uniformity in reporting makes it very difficult to perform comparisons and aggregation.

Limited role of surveillance and reporting system

Hospital play a major role in the surveillance system via records and reporting statistics. Information obtained from clinics are limited and inadequate, most especially from the private sector. In Africa and South Asia for instance, case notifications are barely enforced and hence number of cases mostly unknown. In the Americas, generally, surveillance has reported to be is passive and ineffective (WHO 2009a).

Misclassification in reporting of dengue

The difficulty in the WHO classification system of cases and unfamiliarity with cases of dengue are two main major contributory factors for misclassification (Cechenil et al., 2016, WHO 2012). In a one year review of medical records in Puerto Rico, out of 88 and 14 patients who had DHF and DSS respectively, only 17 individuals with DHF and 3 with DSS with identified (Noyd & Sharp, 2015). This suggest that severe cases of dengue were five times more reported during the review than were reported during the routine surveillance. There is therefore the need to address resource problem in order to better recognized the disease severity and improve on reporting. In another review in Taiwan, 71% supposedly DHF patients were when cases were not actually cases severe dengue upon review (Yeh et al., 2017).

Global Strategy for Prevention and Control.

The alarming trends of dengue over the years led to the implementation of an 8-year global strategy from 2012 to 2020 by WHO. The strategy aims at moving from reactive response to proactive risk assessment, early warning systems and preventive measures which are guided by entomological and epidemiological surveillance. The strategy also emphasizes on building capacity to increase resilience of future outbreak. Specifically, the strategy seeks to reduce dengue mortality by at least 50% by the year 2020, reduce morbidity by at least 25% by 2020 and finally estimate the true burden of the disease by 2015. In 2017, WHO reported that, there was 53% reduction of dengue cases which implies a significant downward trend of cases.

The target stakeholders of this strategy include leader of the national control programmes, urban planners, researchers and funding agencies and water resource managers. Again, the strategy highlighted in addition to stakeholder's involvement, the inclusion of integrated vector management approach and sustainable control measures. This is very paramount in the achieving of the overall aim within 8-year timeline.

Implementation of the global strategy is underpinned by five factors; advocacy and resource mobilization, partnership, coordination and collaboration, communication to achieve behavioral outcomes, capacity building and monitoring and evaluation. The success of this implementation requires a rigorous effort and engagement by all governments and their relevant sectors.

Advocacy and resource mobilization

International advocacy and funding effort are very minimal in the prevention and control of the disease. Although some research funding organizations are raising funds for research at national and local levels, the effort at the international level is insignificant. This advocacy and funding gap affects international response such as training courses, outbreak response and outbreak preparedness (Morrison et al., 2008). It is therefore imperative that the WHO develops advocacy plans in order to get political and resource mobilization support from governments from its member states through their regional and country offices. In the building and strengthening of advocacy support, high profile public figure should be used to champion the cause of dengue and also make use of regional collaborations. In the South Eastern Asia Region, there is a

regional initiative by the WHO countries to annually observe "dengue day" on the 15th of June (WHO, 2012). Advocacy campaigns should also target public and private sectors including sanitation and related infrastructure.

Partnership, coordination and collaboration

The success of dengue control programmes is underpinned by multisectoral response and preparedness. The application of this approach has not been successful over the years. Countries should therefore endeavor to promote this approach at all levels, most especially, in endemic countries. At the international front, there is the disconnection between organizations and institutions working on dengue research and control. They should therefore promote collaboration, coordination partnership for a more desirable impact. Locally, intersectoral approach which requires the local Ministry of Health and their relevant allies including the private sector non-governmental agencies and the local communities is essential for effective prevention and control (Morrison et al., 2008). Materials, data and human resource sharing is also effective in prevention and control especially in emergency situations where these resources need to be mobilized quickly to alleviate the effect of an epidemic. The high dengue morbidity in urban centres calls for coordination with urban planning and water resource management in addressing the piped drinking water, and sanitation issue in order to reduce the urban breeding sites of the vector through the improvement of drainage at a collection point. Building partners with industries and allied sectors such as water and sanitation can prevent *Aedes sp* infestation in an area. The achievement of the WHO global strategy is also deeply hinged on network

between intersectoral and intra-sectoral collaboration. When facilities and research are networked, it provides a more rigorous approach than independent sectors and also provides a platform for members to resolve both inter and intra-agency issues and share the best practices. Partnership and networks help in effective prevention and control by influencing the strength and synergizing the efforts of partners. In many endemic countries where intersectoral activities is not properly synchronized, it defeats the effort of good surveillance and preventive control measures (World Health Organization, 2011). There is therefore the need to build relationship and interconnected hierarchical structures for control of the infection.

The World Health Organization should therefore support the efforts in harmonizing data exchange, processing and intercountry partnership among its member states.

Communication to achieve behavioural outcomes

Effective communication is very crucial in the effort to prevent and control dengue infection (Harrington et al., 2013). Individuals have a big role to play in this effort to reduce morbidity and mortality through their behavioural modifications. It is very important for the population at risk to adopt protection and reduction behaviours such as keeping environmental hygiene to reduce vector density. In the light of this, WHO (2012) came out with a communication strategy called the Communication for Behavioural Impact (COMBI) which is systematically designed to focus on behavioural communication strategy for modifying behaviours associated with vector-borne diseases including dengue.

The application of COMBI reduction effort includes appropriate use of insecticides, community mobilization, reporting dengue cases and acceptance of dengue vaccine when it becomes recommended (Shepard et al., 2004).

Capacity building

According to WHO (2020), there has been a neglect of capacity at all level within most WHO regions through unsustainable training and collective efforts. Building capacity requires access appropriate equipment, facilities, well-trained staff to effectively execute control and prevention programmes. Building training capacity for medical entomologist, social scientist, public health personnel, communication experts, epidemiologists, disease control officers and medical laboratory staff is also essential in the collective and effective control strategy (Beatty et al., 2010). The WHO dengue endemic regions should also organize frequent in-service training for its staff frequently and not only when there is an epidemic. Efforts must also be made by endemic countries to adapt the WHO dengue guidelines for control programmes such as case detection and management, case definitions, diagnosis, prevention and control for training.

Monitoring and Evaluation

An efficient monitoring and evaluation system are very critical in the efficient implementation of dengue strategy. It helps in the assessment of how effective the strategy is, how well it is working, its challenges and shortcomings and identifies the areas for improvements. World Health Organization (2020) has hinted that, monitoring and evaluation is very weak in nearly all dengue endemic countries

and needs to be strengthened. Studies (Eisen et al., 2009; PAHO, 2018; World Health Organization, 2011) have advised the use of use one national monitoring and evaluation system and team to reduce reporting burden and duplicity of data and enhanced uniform analysis and interpretation. In evaluation and monitoring, there is the need for a standardize indicator. Although according to the World Health Organisation, the best assessment and trends must rely on both suspected cases and data, accurate surveillance should be the peak goal. Monitoring and evaluation team should be made aware that, routine surveillance is advantageous in estimating case incidence spatially and through time. In the light of this, data should therefore be compiled annually to allow for time effect in the myriad of factors that influence dengue prevalence from time to time and place to place. Monitoring and evaluation being a critical key component of dengue surveillance should in their work provide proper guidance, risk assessment and a better outbreak response plan. Finally, WHO should provide a well-coordinated metrics for evaluation for its member states.

Chapter Summary

The focal point of literature reviewed points out that there are several challenges that undermines the effort of knowing the true magnitude of the disease burden and have therefore recommended strategies which includes research of dengue to address some of those challenges. It is in the light of this, that this research framework was developed to highlight on some of the global existing challenges in Cape Coast in order to create awareness of the disease.

CHAPTER 3

MATERIALS AND METHOD

Introduction

This study was a hospital-based cross-sectional study with the aim of determining the seroprevalence of dengue virus and its molecular detection from the University of Cape Coast hospital. Samples were taken and serum obtained through centrifugation. Serum samples were tested for IgG and IgM using rapid diagnostic kit and ELISA. Viral nucleic acid was extracted from seropositive samples and tested for its presence using conventional reverse transcriptase polymerase chain reaction (RT-PCR).

This chapter also describes the study site, study design, population, inclusion and exclusion criteria, sample collection procedures, laboratory protocols and statistical tools used for the analysis of the data. It also emphasizes on ethical consideration.

Study design

The study was a cross-sectional hospital-based study and the sampling method used was convenient sampling. Participants were selected without any randomization so far as they consent and qualify to be included in the study.

Study site

The study was conducted at the University of Cape Coast Hospital in the Cape Coast Metropolis, Central region. This facility provides various health care

services through in-patient and out-patient departments. The population of Cape Coast is about 169,894 according to the census conducted in 2010. The city is located on longitude 1° 15' W and latitude 5° 06' N and occupies approximately 122 square kilometers. Cape Coast is characterized by hot and humid climate which is influenced by the northeast trade wind and southwest monsoon wind. The Metropolis has a double annual rainfall with a mean temperature of 25°C.

The most common means of discarding refuse in Cape Coast is by either dumping in a container (56.7%) or dumping unto an open dumpsite (21.1%) (Service, 2013). In most dwelling places in the city, liquid waste is disposed by either throwing it onto the compound (34.0%) (Ghana Statistical Service, 2013). The Metropolis records 66.7% of houses in urban areas which serve as a home to 75% of household population. Averagely, there are 2.3 households per house in Cape Coast as compared to a regional and national average of 1.5 and 1.6 respectively (Ghana Statiscal Service, 2013).

Finally, Cape Coast has tourist attraction sites hence attracts a large number of tourists globally and locally.

Population

The research was conducted among patients who were seeking routine medical care at inpatient and outpatient department of University of Cape Coast Hospital. These patients included both males and females who were 18 years and above and at least presented with three malaria-like symptoms. Participants were selected based on inclusion and exclusion criteria.

Inclusion criteria

Individuals included in this study were 18 years and above presenting with at least three symptoms of the following: fever, myalgia, arthralgia, rash, headache, abdominal pain, retro-orbital or ocular pain and haemorrhagic manifestation (i.e. petechiae, purpura, epistaxis, gum bleeding, etc.). According to WHO, individuals who present with at least three of the above should be screened for dengue viral infection.

Exclusion criteria

The study excluded patients who visited the health facility who did not present with at least three of the symptoms mentioned above, people below age eighteen, and those who were not willing to partake in the study.

Sample size

The minimum sample size was determined by the formula (Cochran).

$S = \underline{Z^2 (P) (1-P) /}(ERROR)^2$

Where Z, 1.96 is the standard normal variate at 5% type error, ($P<0.05$) it is for the confidence interval of 95%).

Minimum sample size, $S = \underline{1.96^2(0.21) (1-0.21} / (5/100)^2$

$= 254.9 = 255$ clinical sample

Using 5% non-responsive rate, 270 participants were enrolled.

Data collection instrument

A structured questionnaire was used to obtain information on demographics, clinical signs, and symptoms, and risk factors after participant had agreed to voluntarily take part in the study.

Blood sample collection

A trained phlebotomist collected 3-4ml/kg of venous blood samples aseptically from the study participants using sterile needle and syringe or vacutainer needle into a plain tube. The venous blood was collected from the median cubital vein of the left arm. Sample identification number 001-270 was labelled on the plain tubes accordingly from the first participant to the last participant.

Sample handling and storage

The blood samples transported from the hospital to the laboratory were spun at 300 x g for 5 minutes at 4°C. A sterile disposable transfer pipette was used to transfer serum into a sterile 1.5 ml Eppendorf tube and stored at -20°C until testing.

Laboratory Procedure

Rapid Diagnostic Testing for IgG and IgM

Serum kept at -20°C and RDT kits (Voyage medicals, One Step, China) kept in 4-8°C refrigerator was brought to room temperature before testing as instructed by the manufacturer. The test kit was labeled with the specimen ID number. Fifty microliter pipette was filled with serum and holding the dropper vertically, 50µl

serum was dispensed into the sample wells (IgG and IgM) whiles ensuring that there were no air bubbles. With a timer set, results were read within 20 minutes.

Interpretation of results

For a negative result, only the control pink band appeared on the test region of the cassette. For a positive result, three pink bands; control, IgG, and IgM bands or two pink bands; control and IgG or IgM appeared on test region of the cassette indicating that the specimen contained detectable amount of anti-dengue IgG and IgM. A test was considered invalid if a coloured band did not appear at control region, indicating a possible error in performing the test, hence, test was repeated until gave a valid result.

Dengue IgM ELISA

All samples were brought to room temperature and tested for anti-dengue IgM/IgG using ELISA kits from DIAsource Immunoassays S.A, Belgium. Serum of 10µl was diluted in 1000µl of sample diluent (1:100). It was then mixed carefully by vortexing. Fifty microliters (50µl) of neutralising reagent was added to all wells except for well A1 and the control wells. Hundred microliters of positive control and negative control were dispensed in the positive and negative control well in duplicate. Finally, 100µl of diluted samples were added to each well. The microplate was then incubated for 60 minutes at a temperature of 37°C. After incubation, the microwells were washed with a wash buffer solution for five cycles (aspiration + dispensing of 350µl per well=1 cycle). Plates were soaked for 20-30 seconds between cycles. Hundred microliters of enzyme conjugate were

pipetted into each well except the blank well and covered with a sealer. The microplate was incubated for 60 minutes at 37°C. Microwells were washed again after the incubation period. After washing, 100µl of the chromogenic substrate mixture was pipetted into each well including the blank well. The microplate was then incubated at room temperature (25°C) for 20 minutes in the dark. Sulphuric acid (100µl) was pipetted into all the wells. The addition of the acid turned all positive controls and all positive samples from blue to yellow. The colour intensity of the solution in each well was measured at an optical density (OD) of 450nm blanking A1 well.

Interpretation of Results

Cut-Off=NC+0.250; where NC is the mean OD450nm value of the negative control. The test results are interpreted as a ratio of sample OD450nm value (S) and the Cut-Off value (Co) or S/Co

Table 1: Interpretation of results according to manufacturer

S/Co	Interpretation
<0.9	Negative
0.9-1.1	Equivocal
>1.1	Positive

IgG ELISA

Using ELISA kits from DIAsource Immunoassays S.A, Belgium, serum of 10µl of serum was diluted in 1000µl of sample diluent (1:100). It was then mixed carefully by vortexing and 100µl of positive control and negative control were dispensed in the positive and negative control well in duplicate. Finally, 100µl of

diluted samples were added to each well. The microplate was then incubated for 60 minutes at a temperature of 37°C. After incubation, the microwells were washed with a wash buffer solution for five cycles (aspiration + dispensing of 350µl per well=1 cycle). Soaking time of 20-30 seconds was allowed between cycles. Hundred microliters (100µl) of enzyme conjugate were pipetted into each well except the blank well and covered with a sealer. The microplate was incubated for 60 minutes at 37°C. Microwells were washed again same as described above after the incubation period. After washing, 100µl of the chromogenic substrate mixture was pipetted into each well including the blank well. The microplate was then incubated at room temperature (25°C) for 20 minutes in the dark. Sulphuric acid (100µl) was pipetted into all the wells. The addition of the acid turned all positive controls and all positive samples from blue to yellow. The colour intensity of the solution in each well was measured at an optical density (OD) of 450nm blanking A1 well.

Interpretation of Results

Cut-Off=NC+0.250; where NC is the mean OD450nm value of the negative control.

Samples showing an OD 450nm value lower than the Cut-Off value are considered negative for anti-dengue IgG.

Nucleic acid (RNA) Extraction

Viral RNA was recovered using Quick RNA MiniPrep extraction kit (Zymo Research). Serum samples were lysed by adding 300µl of RNA lysis buffer to

100µl of serum. The supernatant was transferred into a Spin-Away filter in a collection tube and centrifuged at 15,000×g for 30 seconds at room temperature. The flow-through was saved. One volume of ethanol (95%) was added to the flow-through and was mixed by vortexing. The mixture was then transferred to a Spin Column in a collection tube and centrifuged at 15000×g for 30 seconds at room temperature. The flow-through was discarded. The column was treated with DNase 1 by adding 400µl of RNA wash buffer to the column and then centrifuged at 15000×g for 30 seconds at room temperature. A mixture of 5µl of DNase 1 and 75µl of DNA digestion buffer in an RNase free tube was added directly to the column matrix. It was then incubated at room temperature for 15 minutes. After incubation, 400µl of RNA prep buffer was added to the column and centrifuged at the same speed, time and temperature. The flow-through was again discarded. Seven hundred microliters (700µl) of RNA wash buffer was added to the column and centrifuged, and the flow-through was discarded again. To ensure complete removal of the wash buffer, 400µl of wash buffer was added again to the column and centrifuged for 2 minutes. The column was then transferred into an RNase free tube. Column-bound RNA was eluted by adding 100µl RNase-free water directly to the column matrix and centrifuged for 15000×g for 30 seconds at room temperature.

Reverse transcription-polymerase chain reaction (RT-PCR)

The detection of dengue virus RNA antigen was carried out in Biorad T100 thermal cycler using a one-step RT-PCR master mix kit (New England Biolabs Inc, USA**).** The master mix for a single reaction is shown on Table 2. Reverse

transcription was performed using reverse transcriptase for 30 minutes at 45°C. This was followed by initial denaturation step of DNA at 94°C 5 minutes. Then, 40 PCR cycles consisting of denaturation at 94°C for 30 seconds, annealing of primers at 65°C for 30 seconds and extension for 1 minute at 72°C were carried out. This was followed by a final extension at 72°C for 7 minutes. The targeted gene for PCR amplification was the core-pre-membrane (CprM) region using universal dengue primers D1 and D2 (Sangon Biotech, China) which is previously described by Lanciotti et al. in 1992.

PCR product was separated by electrophoresis on a 1.5% agarose gel TAE buffer stained with ethidium bromide. Each well was loaded with 5µl of the PCR product and 3µl of purple 6X DNA loading dye (New England Biolabs, USA). Samples were separated along with a 100bp DNA ladder at 90 volts for 45 minutes. The gel was visualized by ultraviolet fluorescence light using a transilluminator and images were acquired using a phone camera.

Table 2. RT-PCR master mix for a single reaction

COMPONENT	VOLUME (µl)
One Taq One-Step Reaction Mix (2×)	12.5
One Taq One-Step Enzyme Mix (25×)	0.5
10 µM Universal D1 Primer	1
10 µM Universal D2 Primer	1
Nuclease free water	9
RNA template	1

Total volume per reaction	25

Table 3. PCR cycling conditions

CYCLE STEP	TEMPERATURE	TIME	CYCLES
Reverse Transcription	45°C	30 minutes	1
Initial Denaturation	94°C	5 minutes	1
Denaturation	94°C	30 seconds	
Annealing	65°C	30 seconds	40
Extension	72°C	1 minute	
Final Extension	72°C	7 minutes	1
Hold	4°C	∞	

Table 4. List of oligonucleotide primers used: Lanciotti et al (1992)

DENV SEROTYPE	PRIMER NAME	5'-3'OLIGONUCLEOTIDE SEQUENCE	GENOME POSITION	AMPLICON SIZE	PRIMER PAIR
DENV1-4	D1	TCAATATGAAACGCGCGAGAAACCG	134-161	511	D1 and D2
DENV1-4	D2	TTGCACCAACAGTCAATGTCTTCAGGTTC	616-644	511	D1-D2

Statistical Analysis

Descriptive statistics were used to describe demographic data in terms of frequencies, percentages, median and interquartile ranges. Chi-square was used to find the relationship between gender and serostatus, anti-dengue IgG and IgM. When any of the expected value was <5, fisher exact p-value was reported. At a Chi-square significant level of less < 0.05, binary logistic regression was used to find the strength of association between anti-dengue IgG and IgM via odds ratio. A graph of marginal adjusted probability was used to predict the probability of IgM positivity between IgG seronegative and seropositive individuals at a 95% confidence interval level. Cohen's Kappa inter-rater reliability was used to evaluate the diagnostic accuracy of RDT using ELISA as a gold standard for measurement.

Ethical Approval

Ethical clearance was sought from the University of Cape Coast Institutional Review Board. IRB Number: UCCIRB/CHAS2018/20

Chapter Summary

This chapter described in detail the methodological approach and research methods used in this study. It emphasized on ethical issues as well as detailed laboratory protocols employed. The chapter described fully the various steps used in each protocol.

The methodology had a limitation. The flavivirus group of viruses have a similar genome hence cross-react with each other. There was therefore the need to confirm samples that tested positive for IgG and IgM with the plaque neutralization reduction test (PRNT); this was not done due to its unavailability.

CHAPTER 4

RESULTS AND DISCUSSION

Introduction

This chapter describes the demographic characteristics of participants in terms of age, sex, and educational status. It shows the frequencies of seropositive samples by ELISA. It also shows the relationship between gender and serostatus by the use of Chi-square. Cohen's Kappa interrater reliability measurement of agreement was used to explore the performance characteristics of RDT kit. It also highlights on the seroprevalence values by geographical location and also shows PCR results.

General characteristics of participants

General characteristics of the data was analyzed to determine the frequency and percentage distributions of participants in terms of age, gender, and their educational background. From the results analyzed, the median age was 31. In terms of gender distribution, out of 270 participants, 103 (38.1%) were males and 167 (61.9) were females. Majority of the participants; 139(51.1%) had had tertiary education. Those with senior high school/vocational-technical/form were 4 representing 24.1%. Participants with junior high school and primary education were 37 and 5 representing 13.7% and 1.85% respectively. Finally, individuals with no formal education were 24 representing 8.8%. (Table 5)

Table 5: Demographic Characteristics of participants

Variable	m(IQR)	n(%)
Age	31 (23-43)	
Age category		
18-28		106 (39.26)
29-39		77(28.52)
40-49		33(12.22)
50-59		35(12.96)
60-69		11(4.07)
70-79		7(2.59)
80-89		1(0.37)
Sex		
male		103 (38.15)
female		167 (61.85)
Educational status		
Non-formal		24 (8.8)
Primary		5 (1.85)
JHS		37 (13.7)
SHS/Votec		65 (24.07)
Tertiary		139 (51.48)

N=270

Seroprevalence variations among participants demographic characteristics

The overall seroprevalence in this study was 12.96% (IgG and IgM combined). The seroprevalence for IgG was 12.6% whiles IgM was 2.2%. In this study, the seropositivity rate in females were higher in females than males for both IgG and IgM. The age group with the highest IgG seropositivity rate were those between ages 29 and 39. This same group recorded the highest seroprevalence rate in terms of current exposure (IgM). The only age group that did not show any positivity in terms of IgG and IgM participants between ages 80-89. Participants who were attending the University hospital from Elmina recorded the highest IgG (3.3%)

and IgM (1.85%) seropositivity rate as compared to those from other towns. Apewosika also recorded an IgM seropositivity rate of 0.37%. Apewosika and Elmina were the only towns where IgM antibodies were detected among participants. (Table 6)

Table 6. Seroprevalence of demographic variables and location

	seroprevalence (%)	
Variable	IgG(%)	IgM(%)
Overall	12.6	2.2
Gender		
male	5.1	0.74
female	7.41	1.48
Age		
18-28	2.59	
29-39	4.07	1.11
40-49	2.59	0.74
50-59	2.22	0.37
60-69	0.74	
70-79	0.37	
80-89		
Location		
Elmina	3.3	1.85
Akotokyir	0.37	
Abura	1.48	
Kotokuraba	1.85	
Apewosika	1.9	0.37
UCC	2.6	
Kingsway	1.11	

N=270

Past and current exposure to dengue by gender

Analysis of past and current exposure to dengue by gender difference was done to ascertain gender variability to dengue exposure. The results demonstrated high proportions of past and current infection in females than males. As it can be seen on the frequencies cross tabulated on Table 7, there was no significant difference

between gender and past exposure (IgG), $\chi 2$ (N=1,270) =0.15, p=0.69. This was also the same for the relationship between gender and current exposure (IgM), $\chi 2$ (N=1,270) = 0.06, p=0.8.

Table 7: A table showing a chi-square association between gender and IgG and IgM

	IgM				IgG			
Sex	Negative	Positive	X^2	p-value	Negative	Positive	X^2	p-value
Males	101	2	0.06	0.81	89	14	0.15	0.697
Female	163	4			147	20		
Total	264	6			236	34		

Seroprevalence by Primary and Secondary exposure

Table 8 shows the dynamic between IgG and IgM in terms of primary and secondary dengue exposures. The seroprevalence of participants with recent primary exposure (IgG-IgM+) were 0.37%. Those with recent secondary infection (IgG+IgM+) were 1.85%. The seroprevalence of past primary infection (IgG+IgM-) or probable secondary infection was 10.74%. Two hundred and thirty-five (235) representing 87.04% did not show any evidence of dengue infection (IgG-IgM-).

Table 8. Relationship between IgM and IgG antibody results

Serological group	IgM	IgG	n	Seropositivity rate (%)	Interpretation
1	+	-	1	0.37	Recent/primary infection
2	+	+	5	1.85	Recent secondary infection
3	-	+	29	10.74	Past infection
4	-	-	235	87.04	No evidence of current/past exposure

Diagnostic accuracy of RDT

The objective 2 of this study sought to ascertain the diagnostic accuracy of a commercial RDT kit. The results shown in Table 9 shows the diagnostic parameters of RDT (One step) using ELISA as a gold standard. This analysis was subjected to Cohen's Kappa inter-rater reliability statistical tool. The Cohn's Kappa coefficient value was 0.373 and 0.0001 for IgG and IgM respectively indicating poor to fair agreement between the two tests for test anti-dengue IgG and very poor to no agreement between the two tests for anti-dengue IgM. The Kappa values suggested a poor RDT performance for both IgG and IgM hence were not used in the analysis of this study except to show it is seropositive and negative frequency distribution and its diagnostic accuracy.

Table 9. Performance characteristics of RDT

Antibody	Performance of RDT	Percentage
IgG	Sensitivity	26.5%
	Specificity	99.6%
	Positive predictive value	90%
	Negative predictive value	90.4%
IgM	Sensitivity	0
	Specificity	100%
	Positive predictive value	0
	Negative predictive value	97.8%

Cohen's Kappa measurement of agreement value for IgG=0.373, Kappa value for IgM=0.0001

Gel electrophoresis results of RT-PCR products

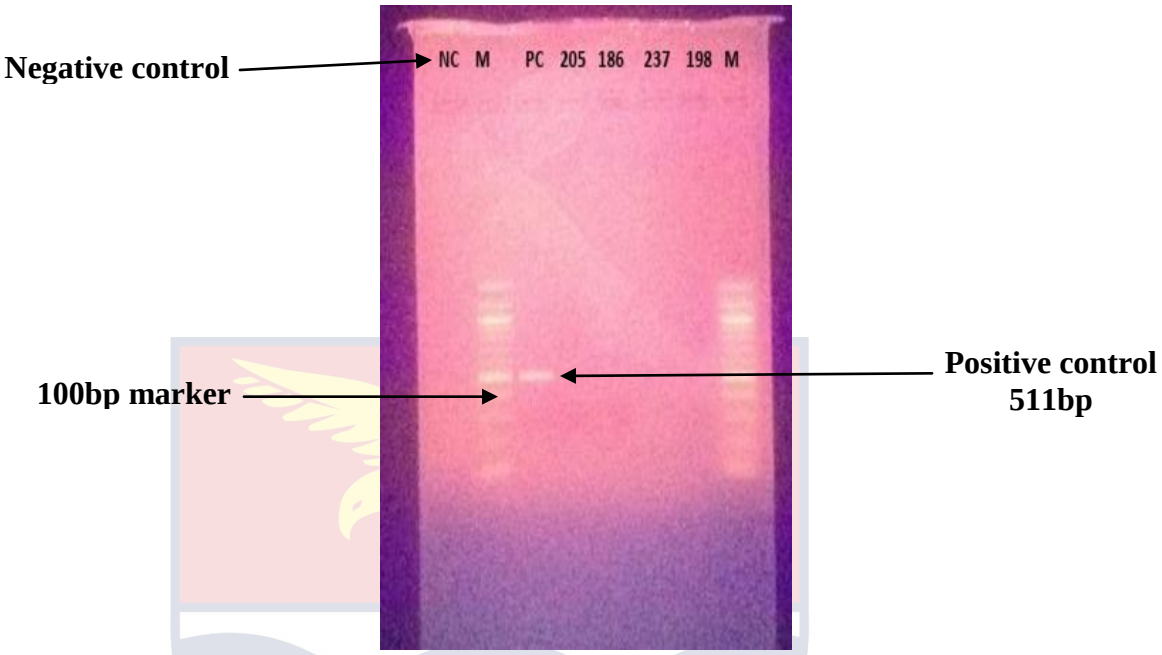

Figure 3A. Gel Image of four samples (figures), positive and negative controls and a 100 base pair marker.

In figure 3A, sample numbers 205 and 198 tested positive for both IgG and IgM whiles sample numbers 205 and 237 tested positive for only IgG.

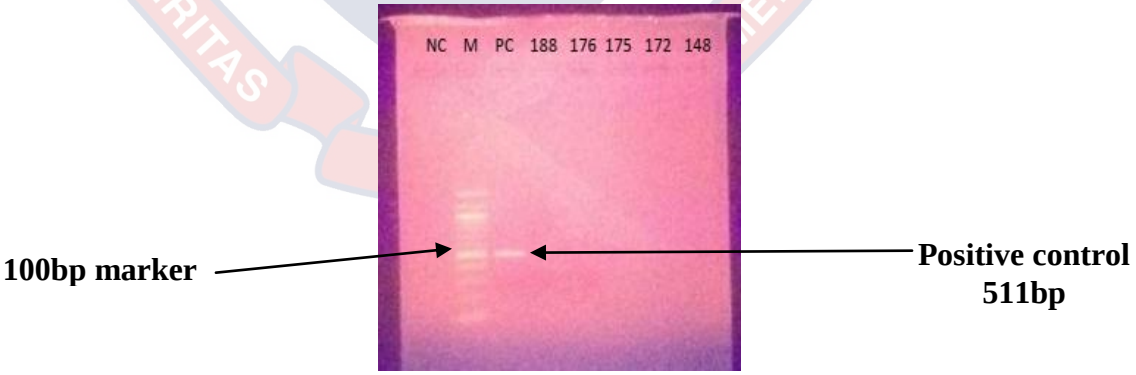

Figure 3B. Gel image showing sample numbers, marker and positive control band

Sample numbers 188, 176, 175, 172 and 148 tested positive for IgG alone

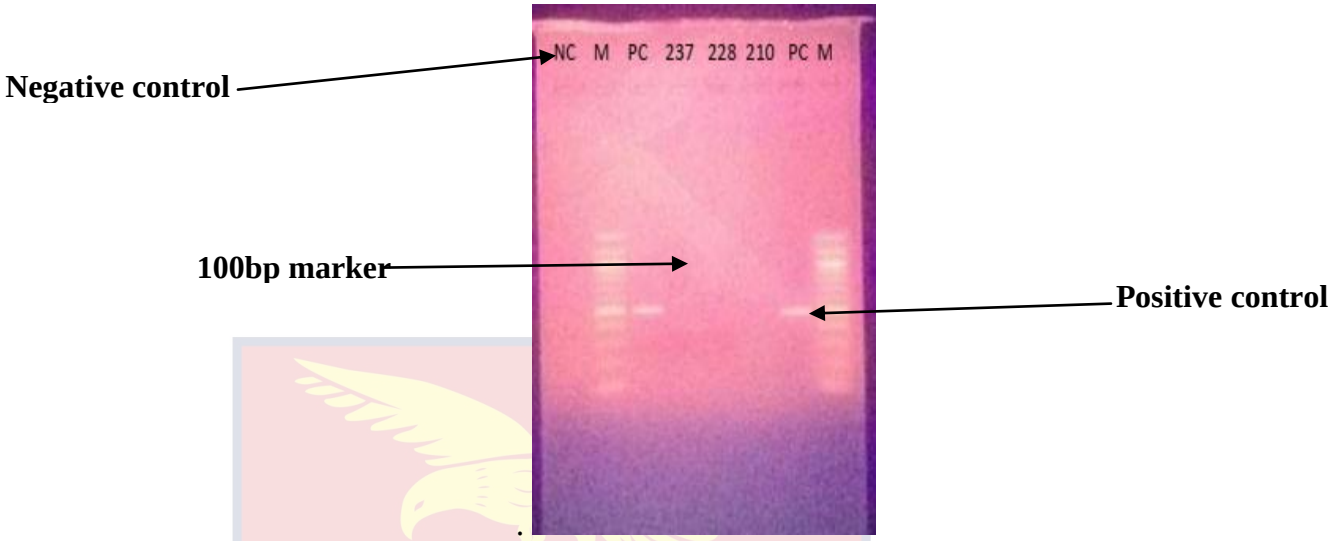

Figure 3C. Gel image showing sample numbers, marker and positive control band

Figure 3C shows a gel with negative control denoted as NC, 100 base pair DNA ladder denoted as M and positive control denoted as PC. Samples 288 and 237 were IgG positive whiles sample 210 tested positive for both IgG and IgM.

The conventional RT-PCR did not show any RNA positivity hence all samples that were tested by PCR were negative.

DISCUSSION

This study sought to determine seroprevalence, assess diagnostic accuracy of RDT, isolate dengue virus and determine the geographical distribution of circulating antibodies and virus. The overall seroprevalence of dengue was 12.9%. Seroprevalence for IgG and IgM were 12.6% and 2.2% respectively. On gender, females recorded a high seroprevalence (7.4%) than males (5.2%) in terms of past exposure (IgG). On current exposure (IgM), females again recorded a high seroprevalence (1.5%) than males (0.7%). Seroprevalence of individuals with dual positivity ((IgG+IgM+) (acute secondary infection) was 1.9%. Those with primary and recent infection (IgM+IgG-) were 0.4% whiles those with past and probable secondary infection (IgG+IgM-) were 10.7%. Elmina had the highest seropositivity rate for both IgG (3.3%) and IgM (1.85%). Dengue virus was not isolated from seropositive samples. Some studies shared similar findings with this study whiles others contradicted.

Dengue is an important public health problem in the tropics and sub-tropics of the world. The prevalence of dengue has been noted to be increasing worldwide, especially in sub-Saharan Africa (Goswami et al., 2018). In as much as knowing the global or national prevalence of a disease is important, it is also very essential to know the incidence of disease in a localized manner. This enables the identification of hotspot areas amidst the spread of the disease worldwide.

In this study, seroprevalence of IgG and IgM was 12.6% and 2.2% respectively. These findings were similar to recent studies (Stoler *et al.,* 2015, Ofosu Appiah *et al*, 2018) that were carried out from other parts of the country

and appear to vary along the geographical regions of the world. Study conducted by Humphery et al., (2018) in all the regions of Ghana showed a seroprevalence of 57% and 21% for anti-dengue IgG and IgM, respectively. A similar study on yellow fever suspected patients also showed a seroprevalence of 3.6% and 1.9% for anti-dengue IgG and IgM in that order (Ofosu-Appiah et al., 2018). Narkwa et al., (2016) also carried out a similar study in blood donors with seroprevalence of 43.6% for anti-dengue IgG alone. The disparities between these seroprevalence values may be due to different geographical areas where the research was carried out with different climatic conditions that might vary in favouring the existence of the virus. Some studies that recorded high seroprevalence values (Narkwa *et al.*, 2016) were carried out in rain forest and humid area which favours the survival of the vector.

The variation in frequencies in the incidence of dengue by gender has been attributed to the differences in exposures to the virus such as time away from home (Anker & Arima, 2011). Gender-related factors can also shift over the course of human lifespan and this will affect sexes in contrasting dengue viral infection. Again, gender-related factors can differ across societies and countries. In this study, the seroprevalence was higher in females (8.88%) than males (5.92%). This observation is in contrast with studies conducted in Agogo, Kintampo, Techiman and other parts of Ghana by (Narkwa et al., 2016 and Ofosu-Appiah et al., 2018). Again, in India, (Chitkara et al., 2018) also found a seroprevalence of 24.9% among febrile patients of which males were twice more affected than females. However, a higher female than male seroprevalence was

reported by (Bello et al., 2016) and (Sule et al., 2019) in Nigeria, which was similar to this study. The variability in gender prevalence could also be ascribed to sample size variation because females were almost twice as the males in the study population. Again, the high seroprevalence in female than male in this study could be attributed to the fact that females were more likely to remain at home and engage in domestic activities during the day where the vector is most active.

Positivity of immunoglobulin M with a negative IgG ($IgM^{+}IgG^{-}$) in an individual denotes a recent or primary infection. This implies that this individual is susceptible to a secondary heterologous infection by a different serotype but have a lifelong immunological protection against a homologous serotype. The implication of those with dual positivity ((IgM+IgG+) suggest that, these individuals may have a secondary acute heterologous serotype but they are clinically healthy because they may not have risk factors such as age, host immune response, strain virulence and serotype and comorbidities such as diabetes, bronchial asthma fulminant hepatitis among others that may be implicated in dengue hemorrhagic fever. Individuals with past primary or probable secondary infection (IgG+IgM-) implies these group of have protection against secondary homologous infection. The group with no evidence of exposure (IgG-IgM-) are susceptible to primary dengue infection by all serotypes because they have not built-up any antibodies against the virus(Lima et al., 2012).

The use of RDTs for serological testing is quite common due to the fact that they are very affordable, readily available, and quick in its operation. The market for RDTs continues to grow largely due to its aforementioned characteristics

without proper regulations by national and even international testing agencies (Garg et al., 2019). The sensitivity and specificity values of these kits corresponded with a study conducted by (Blacksell et al., 2006) which looked at the performance of some commercial RDTs for the diagnosis of dengue infection. Low to high sensitivity and specificity values varying from 6% to 65% and 69% to 100% respectively were shown indicating a varying specificity and sensitivity with the use of RDTs. A similar study also demonstrated low sensitivities ranging from 27.8 to 77.7% (Garg, Garg, Singh & Dhole 2019). However, higher specificities ranging from 50% to 100% were found in some cases. These results were distinctively different from the performance characteristics claimed by the manufacturers. It is therefore suitable that the majority of commercial dengue RDT kits for diagnosis may need to be supported by ELISA as quality control measures for serodiagnosis. The results from this study is also supported by the measurement of agreement values obtained from the Cohen's Kappa inter rater reliability statistical analysis. This confirms the use of ELISA as confirmation test to support the poor diagnostic accuracy of the RDT kit.

Elmina recorded the highest seroprevalence value for both anti-dengue IgG and IgM. It has been demonstrated that 71.6% of households in the municipality use improper sanitation facilities (Ecorys, 2013). Therefore, the expansion of Elmina township needs sustainable sanitation service to match the increasing population. The transmission of dengue can display considerable geographical variability at a small spatial scale with significant variations in incidence in neighboring towns in urban settings (Braga et al., 2010). In the study by Braga

and colleague (2010), it was observed that neighborhoods that were deprived of basic sanitary and hygienic conditions recorded a significantly high seropositive rate as compared to areas with good hygienic measures. Again factors that accounts for differences in the transmission of dengue includes level of urbanization, household risk factor, physical environment and vector ecology (Restrepo et al., 2014; Vanwambeke et al., 2006). Marshes and swamps have been implicated to play a high significant role in dengue vector growth (Sarfraz et al., 2012). The Ministry of Lands and Forestry (1999) and Asomanin (2019) have listed Elmina as one of the coastal areas in Ghana with wetlands. It is likely some of this dengue transmission contributing factors might have accounted for the high seropositivity rate for both anti-dengue IgG and IgM in the Elmina township. This finding is in line with a study conducted in Recife in Brazil which demonstrated higher seroprevalence in dengue in deprived neighbourhood; with lack of access to basic sanitation and also overcrowded (Braga et al., 2010).

It was unfortunate viral RNA was not successfully isolated from the seropositive samples for further studies. Narkwa et al. (2016) and Ofosu-Appiah e al. (2018) also recorded no viral RNA positivity in seropositive samples and may be due to the self-limiting nature of the virus; disappears after 10 days, an average of 5 days of disease or symptoms onset (WHO, 2009b). Other recent studies have also reported that, in primary infection, the virus disappears after 10 days from disease or symptom onset while in secondary infection, it does so after 5 days from disease or symptom onset (Muller et al., 2017b). All these might have accounted for the negativity in the viral nucleic acid among seropositive

individuals. Studies that have compared the detection of the virus in the insect vector and in human suspected cases using the RT-PCR method have shown that the detection of the virus in the vector is highly significant and efficient than in human suspected case and have therefore recommended the detection in the virus in the insect vector (De Souza Leandro et al., 2020).

Chapter Summary

The findings of this study revealed that, females were more exposed to the infection than males. Again, it revealed that, some people have had a dual exposure, others been exposed once while some have never been exposed at all. The RDT used in this study had a poor diagnostic accuracy; WHO has stated this to be one of the major gaps in the knowledge of dengue burden hence need for RDT validation.

CHAPTER 5

SUMMARY, CONCLUSION AND RECOMMENDATION

Conclusion

This study concludes that the seropositivity of IgG and IgM among participants suggest dengue virus may be is circulation in Cape Coast Metropolis and Komenda Edina Eguafo Abirem Municipality. Elmina showed the highest seroprevalence of IgG and IgM. The IgG and IgM dynamics showed that majority of participants did not show any evidence of exposure (IgG-IgM-). Meanwhile some participants had a past primary infection or a probable secondary infection (IgG+IgM-), an acute secondary infection (IgG+IgM+) and a recent primary infection (IgM+IgG-). Finally, the study concludes that the RDT kit had a poor diagnostic accuracy.

Recommendations

This study recommends that the Ghana Health Service should create dengue awareness and resource the hospital laboratories with diagnostic tools and equipment required for the detection and management of the infection. Societies and Non-governmental organizations should also institute programs that will help tackle sanitation issues and decrease the vector ecologies. There should also be strict quality control measures by the appropriate authority to ensure good diagnostic accuracy of diagnostic instruments such as RDT's.

Suggestions for Further Research

Surveillance studies including entomological surveys should be done in the KEEA municipality to assess the true burden of the disease. Participants in this area were the least represented yet recorded the highest seropositivity rate for both IgG and IgM.

REFERENCES

Agarwal, R., Pandey, G., & Yadav, N. K. (2018). Comparison of Rapid Immunochromatographic Kits with ELISA for Detection of Dengue in Acute Febrile Cases in a Tertiary Health Care Centre. *International Journal of Current Microbiology and Applied Sciences*, *7*(10), 1646–1650. doi.org/10.20546/ijcmas.2018.710.187

Amoako, N., Duodu, S., Dennis, F. E., Bonney, J. H. K., Asante, K. P., Ameh, J., Mosi, L., Hayashi, T., Agbosu, E. E., Pratt, D., Operario, D. J., Fields, B., Liu, J., Houpt, E. R., Armah, G. E., Stoler, J., & Awandare, G. A. (2018). *Detection of dengue virus among children with suspected malaria, Accra, Ghana*. *24*(8), 9–12.

Anker, M., & Arima, Y. (2011). Male-female differences in the number of reported incident dengue fever cases in six Asian countries. *Western Pacific Surveillance and Response*, *2*(2), e1–e1. doi.org/10.5365/wpsar.2011.2.1.002

Ayukekbong, J. A., Oyero, O. G., Nnukwu, S. E., Mesumbe, H. N., & Fobisong, C. N. (2017). Value of routine dengue diagnosis in endemic countries. *World Journal of Virology*, *6*(1), 9. doi.org/10.5501/wjv.v6.i1.9

Beatty, M. E., Stone, A., Fitzsimons, D. W., Hanna, J. N., Lam, S. K., Vong, S., Guzman, M. G., Mendez-Galvan, J. F., Halstead, S. B., William Letson, G., Kuritsky, J., Mahoney, R., & Margolis, H. S. (2010). Best practices in dengue surveillance: A report from the asia-pacific and americas dengue

prevention boards. *PLoS Neglected Tropical Diseases*, *4*(11). doi.org/10.1371/journal.pntd.0000890

Bello, O. A., Aminu, M., & Jatau, E. D. (2016). Seroprevalence of IgM Antibodies to Dengue Fever Virus among Patients Presenting with Symptoms of Fever in Some Hospitals in Kaduna State, Nigeria. *International Journal of Science and Research (IJSR)*, *5*(3), 1255–1259. doi.org/10.21275/v5i3.nov162015

Bhatt, S., Gething, P. W., Brady, O. J., Messina, J. P., Farlow, A. W., Moyes, C. L., Drake, J. M., Brownstein, J. S., Hoen, A. G., Sankoh, O., Myers, M. F., George, D. B., Jaenisch, T., William Wint, G. R., Simmons, C. P., Scott, T. W., Farrar, J. J., & Hay, S. I. (2013). The global distribution and burden of dengue. *Nature*, *496*(7446), 504–507. doi.org/10.1038/nature12060

Blacksell, S. D., Newton, P. N., Bell, D., Kelley, J., Mammen, M. P., Vaughn, D. W., Wuthiekanun, V., Sungkakum, A., Nisalak, A., & Day, N. P. J. (2006). The Comparative Accuracy of 8 Commercial Rapid Immunochromatographic Assays for the Diagnosis of Acute Dengue Virus Infection. *Clinical Infectious Diseases*, *42*(8), 1127–1134. doi.org/10.1086/501358

Brady, O. J., Gething, P. W., Bhatt, S., Messina, J. P., Brownstein, J. S., Hoen, A. G., Moyes, C. L., Farlow, A. W., Scott, T. W., & Hay, S. I. (2012). Refining the Global Spatial Limits of Dengue Virus Transmission by Evidence-Based Consensus. *PLoS Neglected Tropical Diseases*, *6*(8). doi.org/10.1371/journal.pntd.0001760

Braga, C., Luna, C. F., Martelli, C. M. T., Souza, W. V. de, Cordeiro, M. T., Alexander, N., Albuquerque, M. de F. P. M. de, Júnior, J. C. S., & Marques, E. T. (2010). Seroprevalence and risk factors for dengue infection in socio-economically distinct areas of Recife, Brazil. *Acta Tropica*, *113*(3), 234–240. doi.org/10.1016/j.actatropica.2009.10.021

Caron, Melanie, Grard, G., Paupy, C., Mombo, I. M., Bikie Bi Nso, B., Kassa Kassa, F. R., Nkoghe, D., & Leroy, E. M. (2013). First Evidence of Simultaneous Circulation of Three Different Dengue Virus Serotypes in Africa. *PLoS ONE*, *8*(10), 1–9. doi.org/10.1371/journal.pone.0078030

Caron, Mélanie, Paupy, C., Grard, G., Becquart, P., Mombo, I., Nso, B. B. B., Kassa Kassa, F., Nkoghe, D., & Leroy, E. M. (2012). Recent introduction and rapid dissemination of chikungunya virus and dengue virus serotype 2 associated with human and mosquito coinfections in gabon, central africa. *Clinical Infectious Diseases*, *55*(6), 45–53. doi.org/10.1093/cid/cis530

Cechinel, L. R., Basso, C. G., Bertoldi, K., Schallenberger, B., Ferreira de Meireles, L. C., & Rodrigues Siqueira, I. (2016). Interlibrary Loans and Journal Article Requests adulthood. *Behavioural Brain Research*, *313*, 82–87.

Changal, K. H., Raina, A. H., Raina, A., Raina, M., Bashir, R., Latief, M., Mir, T., & Changal, Q. H. (2016). Differentiating secondary from primary dengue using IgG to IgM ratio in early dengue: An observational hospital based clinico-serological study from North India. *BMC Infectious Diseases*, 1–7. doi.org/10.1186/s12879-016-2053-6

Chitkara, S., Chhina, D., Gupta, V., Mahajan, R., & Sharma, D. (2018). Epidemiology of Dengue Fever among clinically Suspected Febrile Patients at A Tertiary Care Center in Punjab. *Journal of Microbiology and Infectious Diseases*, *8*(February 2017), 43–48. doi.org/10.5799/jmid.434590

De Souza Leandro, A., Da Silva Britto, A., Rios, J. A., Galvão, S. R., Kafka, R., De Oliveira, W. F., Neto, O. F., Silva, I., Delai, R. M., Gonçalves, D. D., Svoboda, W. K., Rivas, A. V., Lopes, R. D., Trench, F. J. P., De Castro, W. A. C., Sibim, A. C., De Oliveira Ribas, L. F., Gois, F. R., Da Costa Vieira, R. F., & Biondo, A. W. (2020). Molecular Detection of Dengue Virus in Mosquitoes as an Early Indicator to Aid in the Prevention of Human Infection in Endemic Areas. *Vector-Borne and Zoonotic Diseases*, *20*(1), 54–59. doi.org/10.1089/vbz.2018.2411

Eisen, L., Beaty, B. J., Morrison, A. C., & Scott, T. W. (2009). Proactive Vector Control Strategies and Improved Monitoring and Evaluation Practices for Dengue Prevention. *Journal of Medical Entomology*, *46*(6), 1245–1255. doi.org/10.1603/033.046.0601

Garg, A., Garg, J., Singh, D. V., & Dhole, T. N. (2019). *Can rapid dengue diagnostic kits be trusted? A comparative study of commercially available rapid kits for serodiagnosis of dengue fever*. doi.org/10.4103/JLP.JLP

Goswami, L., Runumi, C., & Rasul, E. S. (2018). Seroprevalence of Dengue Infection in a Tertiary Care Hospital in Assam. *International Journal of*

Medical and Dental Sciences, *7*(1), 1582. doi.org/10.18311/ijmds/2018/18905

Gubler, D. J. (2012). The economic burden of dengue. *American Journal of Tropical Medicine and Hygiene*, *86*(5), 743–744. doi.org/10.4269/ajtmh.2012.12-0157

Guo, C., Zhou, Z., Wen, Z., Liu, Y., Zeng, C., Xiao, D., Ou, M., Han, Y., Huang, S., Liu, D., Ye, X., Zou, X., Wu, J., Wang, H., Zeng, E. Y., Jing, C., & Yang, G. (2017). Global Epidemiology of Dengue Outbreaks in 1990–2015: A Systematic Review and Meta-Analysis. *Frontiers in Cellular and Infection Microbiology*, *7*, 1–11. doi.org/10.3389/fcimb.2017.00317

Guy, B., & Almond, J. W. (2008). Towards a dengue vaccine: Progress to date and remaining challenges. *Comparative Immunology, Microbiology and Infectious Diseases*, *31*(2–3), 239–252. doi.org/10.1016/j.cimid.2007.07.011

Hadinegoro, S. R. S. (2012). The revised WHO dengue case classification: Does the system need to be modified? *Paediatrics and International Child Health*, *32*(sup1), 33–38. doi.org/10.1179/2046904712Z.00000000052

Harrington, J., Kroeger, A., Runge-Ranzinger, S., & O'Dempsey, T. (2013). Detecting and responding to a dengue outbreak: Evaluation of existing strategies in country outbreak response planning. *Journal of Tropical Medicine*, *2013*(i). doi.org/10.1155/2013/756832

Holmes, E. C., & Twiddy, S. S. (2003). The origin, emergence and evolutionary genetics of dengue virus. *Infection, Genetics and Evolution*, *3*(1), 19–28. doi.org/10.1016/S1567-1348(03)00004-2

Hung, T. M., Clapham, H. E., Bettis, A. A., Cuong, H. Q., Thwaites, G. E., Wills, B. A., Boni, M. F., & Turner, H. C. (2018). The Estimates of the Health and Economic Burden of Dengue in Vietnam. *Trends in Parasitology*, *34*(10), 904–918.doi.org/10.1016/j.pt.2018.07.007

Iguchi, J. A., Seposo, X. T., & Honda, Y. (2018). Meteorological factors affecting dengue incidence in Davao, Philippines. *BMC Public Health*, *18*(1), 1–10. doi.org/10.1186/s12889-018-5532-4

LaRocque, R. C., Breiman, R. F., Ari, M. D., Morey, R. E., Janan, F. A., Hayes, J. M., Hossain, M. A., Brooks, W. A., & Levett, P. N. (2005). Leptospirosis during dengue outbreak, Bangladesh. *Emerging Infectious Diseases*, *11*(5), 766–769. doi.org/10.3201/eid1105.041212

Lim, J. K., Carabali, M., Lee, J. S., Lee, K. S., Namkung, S., Lim, S. K., Ridde, V., Fernandes, J., Lell, B., Matendechero, S. H., Esen, M., Andia, E., Oyembo, N., Barro, A., Bonnet, E., Njenga, S. M., Agnandji, S. T., Yaro, S., Alexander, N., & Yoon, I. K. (2018a). Evaluating dengue burden in Africa in passive fever surveillance and seroprevalence studies: Protocol of field studies of the Dengue Vaccine Initiative. *BMJ Open*, *8*(1), 1–14. doi.org/10.1136/bmjopen-2017-017673

Lim, J. K., Carabali, M., Lee, J. S., Lee, K. S., Namkung, S., Lim, S. K., Ridde, V., Fernandes, J., Lell, B., Matendechero, S. H., Esen, M., Andia, E.,

Oyembo, N., Barro, A., Bonnet, E., Njenga, S. M., Agnandji, S. T., Yaro, S., Alexander, N., & Yoon, I. K. (2018b). Evaluating dengue burden in Africa in passive fever surveillance and seroprevalence studies: Protocol of field studies of the Dengue Vaccine Initiative. *BMJ Open*, *8*(1), 1–14. doi.org/10.1136/bmjopen-2017-017673

Lima, J. R. C., Rouquayrol, M. Z., Callado, M. R. M., Guedes, M. I. F., & Pessoa, C. (2012). Interpretation of the presence of IgM and IgG antibodies in a rapid test for dengue: Analysis of dengue antibody prevalence in Fortaleza City in the 20th year of the epidemic. *Revista Da Sociedade Brasileira de Medicina Tropical*, *45*(2), 163–167.doi.org/10.1590/s0037-86822012000200005

Messer, W. B., Gubler, D. J., Harris, E., Sivananthan, K., & De Silva, A. M. (2003). Emergence and global spread of a dengue serotype 3, subtype III virus. *Emerging Infectious Diseases*, *9*(7), 800–809. doi.org/10.3201/eid0907.030038

Messina, J. P., Brady, O. J., Scott, T. W., Zou, C., Pigott, D. M., Duda, K. A., Bhatt, S., Katzelnick, L., Howes, R. E., Battle, K. E., Simmons, C. P., & Hay, S. I. (2014). Global spread of dengue virus types: Mapping the 70-year history. *Trends in Microbiology*, *22*(3), 138–146. doi.org/10.1016/j.tim.2013.12.011

Morrison, A. C., Zielinski-Gutierrez, E., Scott, T. W., & Rosenberg, R. (2008). Defining Challenges and Proposing Solutions for Control of the Virus

Vector Aedes aegypti. *PLOS Medicine*, *5*(3), e68.doi.org/10.1371/journal.pmed.0050068

Muller, D. A., Depelsenaire, A. C. I., & Young, P. R. (2017a). Clinical and laboratory diagnosis of dengue virus infection. *Journal of Infectious Diseases*, *215*(Suppl 2), S89–S95. doi.org/10.1093/infdis/jiw649

Muller, D. A., Depelsenaire, A. C. I., & Young, P. R. (2017b). Clinical and laboratory diagnosis of dengue virus infection. *Journal of Infectious Diseases*, *215*(Suppl 2), S89–S95. doi.org/10.1093/infdis/jiw649

Murray, N. E., Quam, M. B., & Wilder-Smith, A. (2013). CLEP-34440-epidemiology-of-dengue—Past—Present-and-future-prospects. *Clinical Epidemiology*, *5*, 299–309. doi.org/10.2147/CLEP.S34440

Narkwa, P. W., Mutocheluh, M., Kwofie, T. B., Owusu, M., Annan, A., Ali, I., & Boamah, K. (2016). *Dengue virus exposure among blood donors in Ghana*. *5*, 30–35.

Noyd, D. H., & Sharp, T. M. (2015). Recent Advances in Dengue: Relevance to Puerto Rico. *Puerto Rico Health Sciences Journal*, *34*(2), 65.

Ofosu-Appiah, L., Kutame, R., Ayensu, B., Bonney, J., Boateng, G., Adade, R., Opare, D., & Odoom, J. (2018). Detection of Dengue Virus in Samples from Suspected Yellow Fever Cases in Ghana. *Microbiology Research Journal International*, *24*(1), 1–10. doi.org/10.9734/mrji/2018/41090

PAHO. (2018). *Integrated Management Strategy for Dengue Prevention and Control in the Region of the Americas; 2018*. 70.

Parks, W., Lloyd, L., & UNDP/World Bank/WHO. (2004). *Planning social mobilization and communication for dengue fever prevention and control: A step-by-step guide*. 138 p.

Peck, M. D. (2011). Epidemiology of burns throughout the world. Part I: Distribution and risk factors. *Burns*, *37*(7), 1087–1100. doi.org/10.1016/j.burns.2011.06.005

Raafat, N., Blacksell, S. D., & Maude, R. J. (2019). A review of dengue diagnostics and implications for surveillance and control. *Transactions of The Royal Society of Tropical Medicine and Hygiene*, 1–8. doi.org/10.1093/trstmh/trz068

Restrepo, A. C., Baker, P., & Clements, A. C. A. (2014). National spatial and temporal patterns of notified dengue cases, Colombia 2007-2010. *Tropical Medicine and International Health*, *19*(7), 863–871. doi.org/10.1111/tmi.12325

Rico-Hesse, R., Harrison, L. M., Salas, R. A., Tovar, D., Nisalak, A., Ramos, C., Boshell, J., De Mesa, M. T. R., Nogueira, R. M. R., & Rosa, A. T. Da. (1997). Origins of dengue type 2 viruses associated with increased pathogenicity in the Americas. *Virology*, *230*(2), 244–251. doi.org/10.1006/viro.1997.8504

Ridde, V., Agier, I., Bonnet, E., Carabali, M., Dabiré, K. R., Fournet, F., Ly, A., Meda, I. B., & Parra, B. (2016). Presence of three dengue serotypes in Ouagadougou (Burkina Faso): Research and public health implications.

Infectious Diseases of Poverty, *5*(1), 1–13. doi.org/10.1186/s40249-016-0120-2

Sarfraz, M. S., Tripathi, N. K., Tipdecho, T., Thongbu, T., Kerdthong, P., & Souris, M. (2012). Analyzing the spatio-temporal relationship between dengue vector larval density and land-use using factor analysis and spatial ring mapping. *BMC Public Health*, *12*(1), 1–19. doi.org/10.1186/1471-2458-12-853

Schaffner, F., & Mathis, A. (2014). Dengue and dengue vectors in the WHO European region: Past, present, and scenarios for the future. *The Lancet Infectious Diseases*, *14*(12), 1271–1280.doi.org/10.1016/S1473-3099(14)70834-5

Screaton, G., Mongkolsapaya, J., Yacoub, S., & Roberts, C. (2015). New insights into the immunopathology and control of dengue virus infection. *Nature Reviews Immunology*, *15*(12), 745–759. doi.org/10.1038/nri3916

Service, G. S. (2013). *Regional Analytical Report*. 61–70.

Shepard, D. S., Suaya, J. A., Halstead, S. B., Nathan, M. B., Gubler, D. J., Mahoney, R. T., Wang, D. N. C., & Meltzer, M. I. (2004). Cost-effectiveness of a pediatric dengue vaccine. *Vaccine*, *22*(9–10), 1275–1280. https://doi.org/10.1016/j.vaccine.2003.09.019

Stanaway, J. D., Shepard, D. S., Undurraga, E. A., Halasa, Y. A., Coffeng, L. E., Brady, O. J., Hay, S. I., Bedi, N., Bensenor, I. M., Castañeda-Orjuela, C. A., Chuang, T.-W., Gibney, K. B., & Murray, C. J. (2016). The Global Burden of Dengue: An analysis from the Global Burden of Disease Study

2013 Europe PMC Funders Group. *Pakistan Lancet Infect Dis*, *16*(6), 712–723. https://doi.org/10.1016/S1473-3099(16)00026-8

Stoler, J., al Dashti, R., Anto, F., Fobil, J. N., & Awandare, G. A. (2014). Deconstructing "malaria": West Africa as the next front for dengue fever surveillance and control. *Acta Tropica*, *134*(1), 58–65. https://doi.org/10.1016/j.actatropica.2014.02.017

Stoler, J., Delimini, R. K., Kofi Bonney, J. H., Oduro, A. R., Owusu-Agyei, S., Fobil, J. N., & Awandare, G. A. (2015). Evidence of recent dengue exposure among malaria parasite-positive children in three urban centers in Ghana. *American Journal of Tropical Medicine and Hygiene*, *92*(3), 497–500. doi.org/10.4269/ajtmh.14-0678

Sule, W. F., Fadamitan, T. O., Lawal, O. A., Adebimpe, W. O., Opaleye, O. O., & Oluwayelu, D. O. (2019). Probable primary and secondary dengue viral infections and associated host factors among university undergraduates in Osun State, Nigeria. *Alexandria Journal of Medicine*, *55*(1), 25–30. doi.org/10.1080/20905068.2019.1592935

Toledo Romani, M. E., Vanlerberghe, V., Perez, D., Lefevre, P., Ceballos, E., Bandera, D., Baly Gil, A., & Van der Stuyft, P. (2007). Achieving sustainability of community-based dengue control in Santiago de Cuba. *Social Science and Medicine*, *64*(4), 976–988. https://doi.org/10.1016/j.socscimed.2006.10.033

Vanwambeke, S. O., van Bethem, B. H. B., Khantikul, N., Burghoorn-Maas, C., Panart, K., Oskam, L., Lambin, E. F., & Somboon, P. (2006). Multi-level

analyses of spatial and temporal determinants for dengue infection. *International Journal of Health Geographics*, *5*, 1–16. doi.org/10.1186/1476-072X-5-5

Were, F. (2012). The dengue situation in Africa. *Paediatrics and International Child Health*, *32*(sup1), 18–21. doi.org/10.1179/2046904712Z.00000000048

WHO. (2004). *The global burden of disease 2004*.

WHO. (2006). *Scientific Working Group U N I C E F / U N D P / W o r l d B a n k / W H O*.

WHO. (2009a). Dengue: Guidelines for diagnosis, treatment, prevention, and control. *Special Programme for Research and Training in Tropical Diseases*, x, 147. doi.org/WHO/HTM/NTD/DEN/2009.1

WHO. (2009b). Dengue guidelines for diagnosis, treatment, prevention and control. *Deutsche Medizinische Wochenschrift*, *34*(8), 329–330. doi.org/10.1055/s-0029-1186356

WHO. (2012). Global Strategy for Dengue Prevention and Control 2012–2020. *World Health Organiszation*, 43. doi.org//entity/denguecontrol/9789241504034/en/index.html

World Health Organization (Ed.). (2011). *Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever* (Rev. and expanded. ed). World Health Organization Regional Office for South-East Asia.

Xue, F., Zhu, L., Liu, S., Liu, W., Yang, C., Wang, L., & Cai, L. (2017). Long noncoding RNA ADAMTS9-AS2 is regulated by DNA methyltransferase 1 and inhibits the malignant behaviors of non-small cell lung cancer cells. *International Journal of Clinical and Experimental Pathology*, *10*(3), 2599–2608. doi.org/10.1002/jmv

Yeh, C.-Y., Chen, P.-L., Chuang, K.-T., Shu, Y.-C., Chien, Y.-W., Perng, G. C., Ko, W.-C., & Ko, N.-Y. (2017). Symptoms associated with adverse dengue fever prognoses at the time of reporting in the 2015 dengue outbreak in Taiwan. *PLOS Neglected Tropical Diseases*, *11*(12), e0006091.doi.org/10.1371/journal.pntd.0006091

APPENDIX

SECTION A: DEMOGRAPHICS		
VARIABLE	**DESCRIPTION**	**CODE**
Sex	1. Male 2. Female	
Age	Year(s)	
Geographical Location		
Educational Background	1. No formal education 2. Primary 3. JHS 4. SHS 5. Tertiary	
Occupation		
Marital status	1. Single 2. Married 3. Cohabiting	

SECTION B: CLINICAL SYMPTOMS		
Fever	1. Yes 2. No	
Skin Rash	1. Yes 2. No	
Headache	1. Yes 2. No	
Myalgia	1. Yes 2. No	
Arthralgia	1. Yes 2. No	
Abdominal Pain	1. Yes 2. No	
Haemorrhage (petechiae, epistasis, purpura cutaneous haemorrhage etc)	1. Yes 2. No	
Interviewer:	Signature:	Date:

UNIVERSITY OF CAPE COAST

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OMB NO: 0990-0279
IORG #: IORG0009096

6TH DECEMBER, 2018

Ebenezer Aniakwaa-Bonsu
Department of Microbiology and Immunology
University of Cape Coast

Dear Mr Aniakwaa-Bonsu,

ETHICAL CLEARANCE – ID: (UCCIRB/CHAS/2018/20)

The University of Cape Coast Institutional Review Board (UCCIRB) has granted **Provisional Approval** for the implementation of your research protocol titled **Seroprevalence and characterization of dengue viral infection among febrile patients in clinical facilities in the Central and Western Regions of Ghana.** This approval requires that you submit periodic review of the protocol to the Board and a final full review to the UCCIRB on completion of the research. The UCCIRB may observe or cause to be observed procedures and records of the research during and after implementation.

Please note that any modification of the project must be submitted to the UCCIRB for review and approval before its implementation.

You are also required to report all serious adverse events related to this study to the UCCIRB within seven days verbally and fourteen days in writing.

Always quote the protocol identification number in all future correspondence with us in relation to this protocol.

Yours faithfully,

Samuel Asiedu Owusu, PhD
UCCIRB Administrator

ADMINISTRATOR
ITUTIONAL REVIEW BOARD
JNIVERSITY OF CAPE COAST
Date: 12/12/18