SEROPREVALENCE OF DENGUE VIRUS AND ASSOCIATED FACTORS AMONG HEALTHY INDIVIDUALS IN KARU LOCAL GOVERNMENT, NASARAWA STATE, NIGERIA

BY

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A DISSERTATION SUBMITTED TO
THE SCHOOL OF POSTGRADUATE STUDIES
AHMADU BELLO UNIVERSITY ZARIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR
THE AWARD OF MASTER OF PUBLIC HEALTH (MPH)
IN LABORATORY MANAGEMENT

DEPARTMENT OF COMMUNITY MEDICINE
AHMADU BELLO UNIVERSITY
ZARIA-NIGERIA

DECEMBER, 2016
DECLARATION

I declare that the work in this dissertation entitled “Sero-prevalence of dengue virus among healthy individuals was done by me under the supervision of Prof A.T. Olayinka and Dr. F.J. Giwa.

The information from literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at any university.

____________________       ________________
Adama Ahmad Abubakar       Date
CERTIFICATION

This is to certify that the dissertation entitled Seroprevalence of Dengue virus healthy individuals in Karu Local Government area, Nasarawa State Nigeria by Adama Abubakar Ahmad, was supervised by us and meets the regulations governing the award of the degree of Masters in Public Health (MPH) in Laboratory Management of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to my late mother and sister who stood by me in all situations come rain come shine. They were always there for me to show their love, support, advice and encouragement. I pray to Almighty Allah to grant them forgiveness, mercy and admit them in paradise, Amin.
ACKNOWLEDGMENTS

I am grateful and thankful to Almighty Allah who spared my life until this day and gave me the strength, energy and knowhow to conduct this work in good health. Praise is to Allah the lord of all the worlds.

I would like to thank my beloved nephews Abdullahi Adam, Ahmad Rufa‘i Adam, my niece Khadija Abdullahi, and my cousins who have been there for me to support me in my difficult times.

I wish to thank my family member and friends for their support also during my trial times that rendered their support one way or the other during my trial time.

I also wish to show my sincere gratitude and appreciation to my supervisors who guided me and have been patient with me throughout the course of this work in person of Professor Olayinka A.

I would like to show immense gratitude to my second supervisor Dr. F. J. Giwa for her support, encouragement, and suggestions.

I am also showing my sincere appreciation to my program supervisors for carefully reading the work and making necessary criticism, corrections and suggestion to make my work a better one.

I also wish to thank my field site supervisor for the advice and support rendered during the period samples were processed in his laboratory.

Gratitude is also due to friends, classmates and well-wishers while undergoing this project.
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SUMMARY

Dengue is a mosquito borne viral infection that causes flu-like illness which can develop into a potentially lethal complication such as dengue haemorrhagic fever and dengue shock syndrome. Recently global incidence of dengue has grown dramatically putting half of the population at risk. This study aimed at determining the prevalence of dengue infection, identifying most at risk population and other factors for risk among the study population.

A descriptive cross sectional study was conducted among apparently healthy individuals at Karu LGA, Nasarawa State. Questionnaires were administered and samples obtained and tested using ELISA IgG technique. Univariate/bivariate analysis was conducted on the using Epi-info version 7.1.4 and statistical significance determined at P < 0.05.

A total of 354 blood samples were collected. Antibodies against dengue virus IgG was found in 17 (4.8%). A total of 168 females (47.5%) were recruited, 8 (4.8%) of whom were positive. Among 186 (52.5%) males, 9 (4.8%) were positive. Age group with highest sero-prevalence was >45 years, 7 (22.6%). Occupation with the highest number of sero-prevalence was farmers with 8 (18.6%), followed by Civil servant with 2 (11.1%) positives. Those in private sector had 1 (9.0%), the least was in business men with 3 (3.8%). Level of education with the highest number of positive was found among those with informal education with 5 (26.3%), those with tertiary level of education had the least seroprevalence with 2 (6.1%).

There was high seroprevalence of dengue among the study population. Those > 50 years and farmers were identified as being at a higher risk and also keeping waste bin in the house. Therefore, it is recommended to improved surveillance for dengue and inclusion of dengue testing in health facilities for febrile illnesses.

Keywords: Dengue, apparently healthy individuals, seroprevalence, Nasarawa State.
CHAPTER ONE

INTRODUCTION

1.1 Background Information

Dengue is an acute systemic viral infection caused by four serotypes of dengue virus (DENV). It is an arthropod borne infection transmitted through the bite of an infected mosquito mainly A. aegypti and A. albopictus which are the vectors of the disease that transmit it to humans and found mainly in tropical and sub-tropical regions of the world.\textsuperscript{1,2} Recently transmission has increased primarily in urban and semi-urban areas and has become a major international public health concern.\textsuperscript{1,3} Dengue fever (DF) is considered to be endemic in many countries and in all regions of the world including Africa with poor surveillance leading to under diagnosis of the disease.\textsuperscript{4} Dengue virus circulation in the African continent date back to early 19\textsuperscript{th} to 20th century in several countries such as Burkinafaso, Zazibar, Senegal and only the outbreak in Egypt was confirmed with retrospective neutralizing antibody testing in the mid-50s.\textsuperscript{5} Dengue fever having similar symptoms with malaria most often is diagnosed as malaria. Mixed infection also occur i.e. prevalence of dengue-malaria co-infection is high).\textsuperscript{6} Differential diagnosis of fever is therefore necessary for appropriate clinical care to be initiated.

The clinical outcomes of DEN virus infection could vary from asymptomatic infection to mild febrile dengue fever (DF) to severe and life threatening dengue haemorrhagic fever (DHF)/dengue shock syndrome (DSS).\textsuperscript{7,8,9} The four closely related, but antigenically distinct, serotypes do not cross-protect instead cross react in the sense that infection with one of these serotypes provides lifelong immunity to the infecting serotype only and recent studies have shown that dengue serotype 5 have been discovered.\textsuperscript{9}
Therefore, persons can acquire a second dengue infection from a different serotype, and second infections place a person at a greater risk for dengue haemorrhagic fever (DHF) which is the more severe form of the disease.\textsuperscript{3,7,10} Symptoms of infection usually occur 4-7 days after the mosquito bite and typically last 3-10 days.\textsuperscript{4} The early symptoms of dengue infections; High grade fever, headache, fatigue, malaise, nausea, vomiting, these symptoms mimic malaria, typhoid, measles and influenza and are hyper endemic in the environment, rendering the diagnosis of this viral infections very confusing.\textsuperscript{4,9} Yet again health Institutions in Nigeria lack appropriate diagnostic facilities for this group of viruses even with the existence of factors such as human populations, global travel, increased urbanization, incursion of human activity into the new ecosystems, climatic changes, and collapse of vector control and public health programs, all favour the emergence of arboviruses globally.\textsuperscript{1,2,11} The World Health Organization (WHO) estimates that two-fifths of the world’s population is at risk of dengue infection yet no approved vaccine or specific drug to treat dengue, which is not normally fatal but lands many victims in hospital.\textsuperscript{12}

1.2 Problem Statement

Dengue infection as a fast emerging pandemic prone viral disease in many parts of the world makes it the most important mosquito borne disease in the world and a serious disease of public health importance.\textsuperscript{1,13} In 2013 several outbreaks were reported in sub-Saharan African countries indicating rapid spread of the infection. Severe dengue epidemics were experienced by only nine countries before the 1970s, now it has spread to more than 100 countries in WHO regions of America, Eastern Mediterranean, South Europe, Western Pacific and Africa.\textsuperscript{5} Parallel with the number of cases increasing as the disease spreads to new areas, explosive outbreaks are occurring with around 390 million people infected each year.\textsuperscript{14}
The most current estimate of the disease includes 96 million severe cases and approximately 300 million mild or asymptomatic episodes compares with the WHO’s most recent estimate for overall infections of 50-100 million a year.\textsuperscript{3,15} The virus and the vector has the tendency to spread to new geographical region, this makes it the most prevalent vector borne infection.\textsuperscript{4}

Human can infect mosquitos and the mosquito remains infected for the rest of its life which might be days or weeks.\textsuperscript{1,3} And because of the socio-economic status of the developing countries and lack of efficient vector control, makes contact between mosquitos and humans frequent.\textsuperscript{5}

The similarity of symptoms of dengue fever with several viral diseases including malaria is most often misdiagnosed putting the individual at risk of developing DHF/DSS during a secondary infection.\textsuperscript{16} Majority of the medically important mosquito borne viruses belong to three families; Flaviviridae causing dengue (DENV) and yellow fever (YFV), Togaviridae causing chikungunya (CHIKV) and Eastern equine Encephalitis (EEEV) and Bunyaviridae such as California group viruses La crosse virus (LACV), most importantly members of these families have overlap in their distribution, and their life cycle is completed in both vertebrate and mosquito hosts leading to mixed infections of mosquito borne viruses, broadly classified as co-infection and superinfection, which can lead to complication when left undiagnosed and sometimes fatal.\textsuperscript{2,7} The disease affects children the most, and as earlier mentioned immunity to one of the serotypes does not confer immunity to another serotype. Dengue viruses can cause dengue fever (DF), dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS).\textsuperscript{8,9,11,17}

The combination of factors such as rapid globalization, long-standing presence of Aedes mosquitoes, case reports from travellers, and recent sero-prevalence surveys all implicate West Africa as an emerging front for dengue.\textsuperscript{2,5} The disease is likely unrecognized and under reported simply because of low awareness by healthcare workers. Recently the Ebola virus outbreak in
Nigeria was an eye opener that increased the awareness of not only health care workers but the general population on viral haemorrhagic fevers routes of transmission and vectors of the disease. With very little known about the disease incidence and prevalence, this research hope to provide baseline information on dengue virus transmission, vectors, and associated risk factors for the disease in the area of study and also call for more studies on dengue infection in Nigeria.

1.3 Justification

This study will help to investigate about the prevalence of the infection in the area in question and provide baseline information that may prompt further studies. A disease that is pandemic prone spreading fast through a wider geographical area with no specific treatment constitutes a source of concern to public. Having it at the back of our mind that very few laboratories in the country conducts the diagnosis of dengue and it is not routinely done and also there is no systematic surveillance for the infection. This is coupled with large number of mosquitos and poor vector control hence dengue is likely unrecognized and under reported. As many as half or up to 80% of all dengue infected individuals suffer asymptomatic dengue disease and no specific treatment, where transmissibility can occur between mosquitos and humans in the sense that humans can infect mosquitos and vice versa helps to facilitate the rates of infections among population. Mixed infections between flavivuses that could allow exchange of genetic material leading to the evolvement of a new strain of virus may lead to a huge human and economic loss within a community. Therefore, high index of suspicion on febrile illnesses and call for more surveillance activities is highly recommended. All factors that are considered as regional drivers within West Africa for dengue emergence and expansion are occurring. Recently outbreak of dengue occurred in Abuja with all the four serotypes of the virus isolated, while several studies such as sero-prevalence surveys from different parts of the country e.g. Ibadan in the south-west,
Maiduguri North-east, and Kaduna North-central coupled with other factors driving the dengue infection e.g. vector population dynamics, and future human population dynamics have been demonstrated in Nigeria. Flaviviruses and other tropical viruses, particularly dengue, have occasionally been isolated from travellers returning from West Africa dengue contributing to global spread of the disease to areas that are not endemic. Therefore, dengue is a problem of international health concern. Burden of the disease is not known in Africa and the prevalence of the disease is worrying, since dengue has not generally been seen as a major problem on the continent before now. It is time to conduct research to determine the level of dengue activity. Another significant reason to consider to conduct this study are the impact of the disease in Africa, which is being masked by febrile illnesses such as malaria and typhoid, which are more common and other viral illnesses, prevalence of the disease, morbidity and mortality, preventive potentials, public health importance and both epidemic and pandemic potentials. Also dengue infection has proven to be on the increase both locally and internationally with evidenced from previous researches. With the above factors in mind call for more studies on dengue cannot be over emphasized. To also refresh our memories to an outbreak that occurred April 2014 in Abuja Nigeria have shown a growing burden of a mosquito borne viral diseases. Lack of adequate mosquito control, urban population growth, climate change, rain water harvesting, greater air travel, nonexistence of surveillance activities for dengue and lack of reporting have all led to rapid spread of dengue virus, resulting to the increase in the prevalence. The infection could be circulating in other parts of the country as well, without research salient zoonotic transmission of dengue could be occurring within our communities. There is need for more research on the topic to help establish the actual prevalence, the circulating strain, disease vector, risk factors as well as the burden of the disease estimation in the country. Surveillance for these
group of viruses is of paramount importance due to existence of co-infection among the Flaviviruses and a possible existence of a silent zoonotic transmission cycle which could offer a potential mechanism for emergence of dengue in human population.\textsuperscript{22} Hence, this study aimed to determine the seroprevalence of dengue virus antibodies among person of all ages especially in the study area in question will be a stepping stone.

1.4 General and Specific Objectives

1.4.1 General objective

To determine the prevalence of dengue virus antibodies and its associated factors in Karu Local Government Area, Nasarawa State.

1.4.2 Specific objectives

1. To determine the Sero-prevalence of dengue virus antibodies in Karu Local Government Area, Nasarawa State.
2. To determine sero-prevalence rates among different age-groups in the study population.
3. To determine factors associated with the risk of dengue virus infection within the study population.

1.5 Research Questions

1. What is the extent of dengue infection in an apparently healthy population?
2. Is there a difference in seroprevalence among age groups?
3. What factors are associated with dengue infection in this community?
CHAPTER TWO
LITERATURE REVIEW

2.1 Historical Background

Dating back to the ancient times, a lot of epidemics of infectious diseases have occurred in many regions of the world. Infectious diseases such as Black Death, measles, smallpox, syphilis, and cholera have claimed a lot of lives. In recent years, dengue fever (DF) has become a major international health problem affecting tropical and sub-tropical regions around the world, especially in urban and peri-urban areas. The geographic distribution of dengue, the frequency of epidemic cycles, and the number of cases of dengue have increased sharply during the last two decades. In addition, the frequency of a potentially lethal complication of dengue, called dengue hemorrhagic fever (DHF), has begun to occur on a regular basis in countries where only dengue occurred previously. Initially, manifestations include fever, joint pain, abdomen, and musculoskeletal system, and later progress to massive internal and external bleedings in forms of petechiae, ecchymosis, epistaxis, hematemesis, haematuria (haemoglobinuria) with the development of melanoma in the infected person. Several diseases are associated with haemorrhage which includes; Ebola and Marburg viruses, Dengue hemorrhagic fever (DHF) and Yellow fever (YF) and are diseases that are more prevalent in countries with tropical climates, and are the deadliest infectious diseases known. They are transmitted by direct contact with fluids of an infected person or other primate with case fatality rate of approximately 50%. On the other hand, DHF and Yellow fever are transmitted by arthropods and have a variable case fatality rate. Dengue exists in the WHO African Region, even with poor surveillance data, outbreak reports exist, although they are not complete, and there is evidence that dengue outbreaks are increasing in size and frequency. Dengue-like illness has been recorded in Africa.
without laboratory confirmation and could be due to infection with dengue virus or with viruses such as chikungunya that produce similar clinical symptoms.³

Cases of dengue imported from India were detected in the 1980s. In East Africa, the available evidence so far indicates that DEN-1, -2 and -3 appear to be common causes of acute fever. Examples of these are outbreaks in the Comoros in various years (1948, 1984 and 1993, DEN-1 and -2) and Mozambique (1984-1985, DEN-3).²⁰ In western Africa in the 1960s, DEN-1, -2 and -3 were isolated for the first time from samples taken from humans in Nigeria. Subsequent dengue outbreaks have been reported from different countries, example includes Burkina Faso (1982, DEN-2) and Senegal (1999, DEN-2). DEN-2 and DEN-3 cases were confirmed in Côte d’Ivoire in 2006 and 2008. Despite poor surveillance for dengue in Africa, it is clear that epidemic dengue fever caused by all four dengue serotypes has increased dramatically since 1980, with most epidemics occurring in eastern Africa, and to a smaller extent in western Africa, though this situation may be changing in 2008.²³ Many studies conducted recently on the continent on the seroprevalence of dengue have indicated increase on the spread of the virus. In rural area of Western Kenya, a study on seroprevalence of denv-2 sero-complex antibodies was 8.5% by indirect ELISA and 1.2% by PRNT in 2011.²⁵ Another cross sectional study conducted in North-Eastern Tanzania among patient presenting with malaria like illness also found Dengue and Chikungunya co-infection with prevalence of 8.7% based on probable case definition for dengue while that of Chikungunya 3.7% by IgG in 2016.²⁶ Recent studies conducted in Kaduna found a prevalence of 18%²⁷ while another separate study in the same state found prevalence of 51.9% IgM to dengue fever.²⁸ In the north Eastern part of Nigeria several studies have shown that dengue virus is circulating within the community, a seroprevalence of dengue among febrile patient was determined to be 10.2%.¹⁹ Effect of climate as one of the risk factor to dengue
infection have been studied in Maiduguri which concluded that while the transmission of dengue virus may have a general system, its manifestations may differ on a local scale from the global expectation and need for more detailed study on the endemic season of arboviral infection in different part of the country.\textsuperscript{11} While dengue may not appear to be a major public health problem in Africa compared to the widespread incidence of malaria and HIV/AIDS, the increasing frequency and severity of dengue epidemics worldwide calls for a better understanding of the epidemiology of dengue infections with regard to the susceptibility of African populations to dengue and the interference between dengue and the other major communicable diseases of the continent.\textsuperscript{16}

2.2 Dengue Virus

Dengue virus (DENV) is a positive sense single-stranded RNA virus belonging to the Flaviviridae family. DENV genome varies in size from 10.6 to 11 kb and encodes three structural and seven non-structural proteins. The structural proteins are comprised of the capsid (C), membrane (M) and envelope (E) proteins, the non-structural proteins include the NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. DENV genome is flanked by 94 nucleotides (nts) at 5′ untranslated regions (UTR) and 388–462 nts at the 3′ UTR. While the four DENV serotypes share 65–70% sequence homology, they are further clustered into different genotypes due to the high mutation rates. Epidemiological and phylogenetic studies have shown geographical movement and divergence of DENVs.\textsuperscript{1} There are five-strains of the virus, called serotypes, of which the first four are referred to as DENV-1, DENV-2, DENV-3 and DENV-4. The fifth type was announced in 2013. The distinctions between the serotypes are based on their antigenicity.\textsuperscript{4}
2.3 Types of Dengue Fever

The World Health Organization's 2009 classification divides dengue fever into two groups: uncomplicated and severe. Severe dengue is defined as that associated with severe bleeding, severe organ dysfunction, or severe plasma leakage while all other cases are uncomplicated. The 1997 classification divided dengue into undifferentiated fever, dengue fever, and dengue hemorrhagic fever. Dengue hemorrhagic fever was subdivided further into grades I–IV. Grade I is the presence only of easy bruising or a positive tourniquet test in someone with fever, grade II is the presence of spontaneous bleeding into the skin and elsewhere, grade III is the clinical evidence of shock, and grade IV is shock so severe that blood pressure and pulse cannot be detected. Grades III and IV are referred to as "dengue shock syndrome."  

2.4 Epidemiology of Dengue Virus

Dengue is the most rapidly spreading mosquito-borne viral disease in the world. In the last 50 years, incidence has increased 30-fold with increasing geographic expansion to new countries and, in the present decade, from urban to rural settings. An estimated 50 million dengue infections occur annually and approximately 2.5 billion people live in dengue endemic countries. The international health regulation was reviewed in 2005 by WHA that included dengue as an example of a disease that may constitute a public health emergency of international concern with implications for health security due to disruption and rapid epidemic spread beyond national borders. Dengue is common in more than 110 countries. It infects 50 to 528 million people worldwide a year, leading to half a million hospitalizations, and approximately 25,000 deaths. For the decade of the 2000s, 12 countries in Southeast Asia were estimated to have about 3 million infections and 6,000 deaths annually. It is reported in at least 22 countries in Africa; but is likely present in all of them with 20% of the population at risk. This makes it one
of the most common vector-borne diseases worldwide. Infections are most commonly acquired in the urban environment. In recent decades, the expansion of villages, towns and cities in the areas in which it is common, and the increased mobility of people have increased the number of epidemics and circulating viruses. Dengue fever, which was once confined to Southeast Asia, has now spread to Southern China, countries in the Pacific Ocean and America, and might pose a threat to Europe.

Rates of dengue increased 30 fold between 1960 and 2010. This increase is believed to be due to a combination of urbanization, population growth, increased international travel, and global warming. The geographical distribution is around the equator. Of the 2.5 billion people living in areas where it is common 70% are from Asia and the Pacific. An infection with dengue is second only to malaria as a diagnosed cause of fever among travelers returning from the developing world. It is the most common viral disease transmitted by arthropods, and has a disease burden estimated at 1,600 disability-adjusted life years per million population. The World Health Organization counts dengue as one of seventeen neglected tropical diseases.

Like most arboviruses, dengue virus is maintained in nature in cycles that involve preferred blood-sucking vectors and vertebrate hosts. The viruses are maintained in the forests of Southeast Asia and Africa by transmission from female Aedes mosquito species other than A. aegypti to their offspring and to lower primates. In towns and cities, the virus is primarily transmitted by the highly domesticated A. aegypti. In rural settings the virus is transmitted to humans by A. aegypti and other species of Aedes such as A. albopictus. Both these species had expanding ranges in the second half of the 20th century. In all settings the infected lower primates or humans greatly increase the number of circulating dengue viruses, in a process called amplification.
2.5 Pathogenesis of Dengue Infection

The pathogenesis of DHF/DSS is only partially understood. The disease is an immunopathology process, dependent in the vast majority of cases on prior immune sensitization by a heterotypic dengue infection. Infection with one dengue serotype provides lifelong homologous immunity, but only transient cross-protection against other serotypes, making sequential infection possible. The relative risk of experiencing the most severe form of the disease is 100-fold higher after secondary than after a primary infection. The underlying mechanism involves enhanced infection of Fc-receptor bearing monocyte/macrophages by dengue virus complexes to non-neutralizing IgG antibodies. These antibodies are the result of prior infection with a heterologous dengue virus serotype, or, in the case of infants born to immune mothers, the result of waning passive maternal antibody. The infectious immune complexes gain access to Fc-receptor-bearing monocytes more readily than dengue virus alone, with the result that the host has a larger number of infected cells containing quantitatively higher amounts of dengue virus, a phenomenon known as immune enhancement. A second aspect of the pathogenesis is the marked T-cell activation and induction of cross-reactive CD4+ and CD8+ cytotoxic T cells that recognize dengue viral antigens (principally non-structural proteins) on infected monocytes. Although this process is key to the clearance of infected cells and recovery of the host from infection, the result of this interaction in a subset of patients may also have pathophysiological consequences due to release of cytokines with vasoactive or procoagulant properties (interleukins, tumour necrosis factor, platelet-activating factor, and urokinase), complement activation, and release of interferon y. The latter molecule up-regulates expression of Fc receptors and in turn increases antibody-dependent enhancement of dengue virus replication. It is still uncertain what host- and virus-specified factors determine why one individual develops
DHF/DSS and another clears secondary infection without consequence.\textsuperscript{3} Moreover, the precise role of different cytokine mediators in the pathogenesis of DHF/DSS remains to be defined.\textsuperscript{3} Pathogenesis of DHF and DSS is still controversial, the two most frequently theories which are not mutually exclusive to explain the pathogenic changes.\textsuperscript{3}

2.5.1 Secondary infection or immune enhancement hypothesis
Patients experiencing a 2nd infection with heterologous dengue virus (DENV) serotype have significant higher risk for DHF//DSS. Prior infection, through a process of antibody dependent enhancement (ADE), enhances the infection and replication of DENV of the mononuclear cells macrophage. This cells, produce and secrete vasoactive mediators increase vascular permeability hypovolemia and shock.\textsuperscript{35}

2.5.2 Viral virulence theory
Second infection with dengue virus put the patient at risk of dengue haemorrhagic fever, in which the phenomenon is restricted to infection with certain dengue virus strain, or genotype that was initially isolated in South-East Asia. The potential for this strain to produce dengue haemorrhagic fever may be related to their ability to produce greater concentrations of the circulating virus in the blood (level of viremia) or their ability to produce infection in both human and the vector host. Both theories are supported by epidemiologic and laboratory evidence, and are most probably valid.\textsuperscript{36}

2.6 Transmission Cycle
Dengue is transmitted between people by the mosquito \textit{Aedes aegypti} and \textit{Aedes albopictus}, which are found throughout the world. Insects that transmit disease are vectors. Symptom of the infection usually begin 4-7 days after the mosquito bite and last typically 3-10 days. In order for
transmission to occur the mosquitos must feed on a person during a five day period when large amount of the virus are in the blood, this period begins a little before the person becomes symptomatic.\textsuperscript{37} Some people never have significant symptoms but can still infect mosquitoes. After entering the mosquito in the blood meal, the virus will require an additional 8-12 days of incubation before it can be transmitted to another human. The mosquito remains infected for the remainder of its life, which might be days or a few weeks. In rare cases dengue can be transmitted in organ transplant or organ transfusion from infected donors, and there is evidence of transmission from an infected pregnant mother to her foetus. But in the vast majority of infections a mosquito bite is responsible.\textsuperscript{4} In tropical Asia and West Africa, dengue viruses are also transmitted between non-human primates and tree hole breeding mosquitoes, but it is uncertain what relationship, if any, exists between the forest cycle and the circulation of virus between humans and \textit{A. aegypti}.\textsuperscript{38} However, the existence of a completely silent zoonotic transmission cycle affords a potential mechanism for emergence of the disease in human populations and possibly also for selection of virus variants with altered host range and vector relationships. Virus strains representing the forest cycle have been subjected to molecular analysis and found to be distinct, indicating that the forest cycle may be ecologically isolated. An important question for future research is whether the virus strains that circulate in the forest cycle are biologically distinct. Nonhuman primates challenged with strains of dengue virus isolated from humans generally develop abbreviated and significantly lower viremias than humans. These virus strains have not been evaluated for their capacity to induce viremia responses in their natural monkey hosts. It is possible that the apparent separation of forest and human transmission cycles reflects a reciprocal and exclusive adaptation to their hosts (or vectors).\textsuperscript{38}
2.7 Risk Factors for Dengue

There are several factors that drive dengue infection as hypothesize by several experts among which are geographic expansion, climate change, globalization, trade and travel by incidence and reporting to WHO. These potential factors are elaborated below:1,13,39

2.7.1 Climate change factors

Adult vector survival, viral replication and infective periods of vectors all depend on temperature; hence increase of temperature may result in increased survival and or migration of vectors into previously non-endemic geographic areas outside the tropics. Proliferation of Aedes mosquitoes is dependent on climate.40 Weather variability has shown to be predictive of dengue activity, factors such as climate change, mean temperatures are predicted to rise globally which may create climatic and environmental conditions conducive to the proliferation of Aedes species in areas that are currently non-endemic. The climatic suitability of many currently non-endemic areas and climatic similarity with endemic areas suggests that both A. aegypti and albopictus could be established or reestablished in the near future.40

The interrelationship between climate change and other factors may serve as a sufficient casual factor in the current and ongoing expansion of dengue infection; these include multiple factors of the modern world contributing to vector- borne communicable disease. Modern contributing factors to the rapid expansion to the vector-borne communicable disease include globalization, travel and trade, associated with vector accommodating trends in modern human settlement and suitable climate.

2.7.2 Globalization, Travel and Trade

Recent approaches seek to combine climate data with projected social changes, including increased population and economic development in tropical and subtropical areas. While suitable
climate factors are necessary to permit the resurgence and expansion of dengue transmission observed over the last 5 decades, human factors, including increasing global population, urbanization, and socioeconomic constraints on control measures, trends in current human settlement, together with rapidly expanded urban areas, exploding population density, and limited socioeconomic resources, suggest that the human factors in addition to climate factors may be necessary components in understanding current and future risks of dengue transmission. Settlement and socioeconomic factors combine with climate suitably and globalized travel and trade to suggest that human populations and their collective actions strongly contribute to the transmission of dengue to mosquito vectors.  

**Settlement Factors**

Urban and rural settlement patterns, contribute to currently observed trends of increased incidence and expansion of dengue transmission. Rapid urbanization and population growth have been identified as strong contributing factors to the increase of global dengue transmission and geographic expansion. These two factors, particularly in low- and middle-income countries in tropical and subtropical regions, often precede the construction of necessary infrastructures for safe and comprehensive collection, storage, and disposal of water. Urban and suburban development may also provide new man-made breeding sites in the built environment, prior to human inhabitants occupying them. This has been shown even in well planned and financed situations.

**2.7.5 Socioeconomic factors**

Historical dengue incidence and decline in Europe and the US, among other areas, suggests the role of socioeconomic development on dengue transmission and control. Multiple studies compared dengue endemicity and seroprevalence between neighboring border cities in Northern
Mexico and Southern Texas. These highlight the importance of socioeconomic factors on the transmission of dengue, where climatic suitability was similar. Sites from urban areas is recognized as key mechanism to control dengue transmission.\textsuperscript{13}

2.8 Disease

The uncomplicated disease, classical dengue fever, is a biphasic illness beginning abruptly 3-8 days after the bite of an infected mosquito, characterized by fever, headache, and severe malaise, lumbosacral aching, and generalized muscle, joint, or bone pain. Improvement after several days is followed by the reappearance of fever and development of a measles-like rash, generalized lymphadenopathy, and sometimes, minor haemorrhagic phenomena. There are no fatalities and the disease resolves in the second week, although patients may experience prolonged convalescence, with weakness and depression.\textsuperscript{3} Due to the self-limited nature of the infection, little is known about the pathogenesis of classical dengue fever. High titers of virus are present in the blood during the early phase, providing the means for mosquito infection. Dengue virus is predominantly a lymphotropic agent, and the principal target cells for virus replication appear to be mononuclear phagocytes, a fact that assumes greatest relevance in the pathogenesis of DHF/DSS. The onset and early phase of DHF/DSS is identical to that of dengue fever. However, shortly after onset, the patient rapidly deteriorates, developing epigastric pain, restlessness an irritability, thrombocytopenia, and signs of diffuse capillary leakage haemoconcentration, and hypotension. Haemorrhagic manifestations of all kinds occur. In its most severe form (designated DSS, occurring in up to 1/3 of individuals with DHF), patients experience narrowing of the pulse pressure and circulatory failure. The case-fatality rate of DHF/DSS is up to 20\% if untreated, but with supportive treatment consisting of fluid and electrolyte management and oxygen, less than 1\% of such cases prove to be lethal.\textsuperscript{3}
2.9 Dengue Haemorrhagic Fever

The initial phase of dengue haemorrhagic fever is similar to that of dengue fever and other febrile viral illnesses. Shortly after the fever breaks (or sometimes within 24 hours before), signs of plasma leakage appear, along with the development of haemorrhagic symptoms such as bleeding from sites of trauma, gastrointestinal bleeding, and haematuria. Patients may also present with abdominal pain, vomiting, febrile seizures (in children), and a decreased level of consciousness.\textsuperscript{41}

If left untreated, dengue haemorrhagic fever most likely progresses to dengue shock syndrome. Common symptoms in impending shock include abdominal pain, vomiting, and restlessness. Patients also may have symptoms related to circulatory failure.\textsuperscript{41}

2.10 Signs and Symptoms

Many patients with dengue experience a prodrome of chills, erythematous mottling of the skin, and facial flushing, which may last for 2-3 days. Children younger than 15 years usually have a nonspecific febrile syndrome, which may be accompanied by a maculopapular rash. The following symptoms are also experienced by patients.\textsuperscript{10}

Headache, Retro-orbital pain, Severe myalgia: Especially of the lower back, arms, and legs, Arthralgia: Usually of the knees and shoulders, Nausea and vomiting (diarrhoea is rare), Rash: A maculopapular or macular confluent rash over the face, thorax, and flexor surfaces, with islands of skin sparing, Weakness, Altered taste sensation, Anorexia, Sore throat, Mild haemorrhagic manifestations (e.g., petechiae, bleeding gums, epistaxis, menorrhagia, haematuria), and Lymphadenopathy.\textsuperscript{10}
2.11 Dengue Diagnostic Methods

2.11.1 Virus isolation

Specimens for virus isolation should be collected early in the course of the infection, during the period of viremia within the first week of infection. Virus can be recovered may be recovered from serum, plasma, peripheral blood mononuclear cells and attempts may be made from tissues collected at autopsy (e.g. liver, lung, lymph nodes, thymus, bone marrow). Storage: Dengue virus is heat-labile; therefore, specimens awaiting transport to the laboratory should be kept in a refrigerator or stored in a cool box with ice packs. For storage up to 24 hours, specimens should be kept at between +4 °C and +8 °C. For longer storage, specimens should be frozen at -70 °C in a deep-freezer or stored in a liquid nitrogen container. Storage even for short periods at −20 °C is not recommended.4,8

Cell culture is the most widely used method for dengue virus isolation. The mosquito cell line C6/36 (cloned from Ae. albopictus) or AP61 (cell line from Ae. pseudoscutellaris) are the host cells of choice for routine isolation of dengue virus. Since not all wild type dengue viruses induce a cytopathic effect in mosquito cell lines, cell cultures must be screened for specific evidence of infection by an antigen detection immunofluorescence assay using serotype-specific monoclonal antibodies and flavivirus group-reactive or dengue complex-reactive monoclonal antibodies. Several mammalian cell cultures, such as Vero, LLCMK2, and BHK21, may also be used but are less efficient. Virus isolation followed by an immunofluorescence assay for confirmation generally requires 1–2 weeks and is possible only if the specimen is properly transported and stored to preserve the viability of the virus in it. When no other methods are available, clinical specimens may also be inoculated by intracranial route in suckling mice or intra-thoracic inoculation of mosquitoes. New-born animals can develop encephalitis symptoms
but with some dengue strains mice may exhibit no signs of illness. Virus antigen is detected in mouse brain or mosquito head squashes by staining with anti-dengue antibodies.42

- Nucleic acid extraction and purification
- Amplification of the nucleic acid, and detection
- Characterization of the amplified product.

Extraction and purification of viral RNA from the specimen can be done by traditional liquid phase separation methods (e.g. phenol, chloroform) but has been gradually replaced by silica-based commercial kits (beads or columns) that are more reproducible and faster, especially since they can be automated using robotics systems. Many laboratories utilize a nested RT-PCR assay, using universal dengue primers targeting the C/prM region of the genome for an initial reverse transcription and amplification step, followed by a nested PCR amplification that is serotype-specific.8 A combination of the four serotype-specific oligonucleotide primers in a single reaction tube (one-step multiplex RT-PCR) is an interesting alternative to the nested RT-PCR.8

The products of these reactions are separated by electrophoresis on an agarose gel, and the amplification products are visualized as bands of different molecular weights in the agarose gel using ethidium bromide dye, and compared with standard molecular weight markers. In this assay design, dengue serotypes are identified by the size of their bands. Compared to virus isolation, the sensitivity of the RT-PCR methods varies from 80% to 100% and depends on the region of the genome targeted by the primers, the approach used to amplify or detect the PCR products (e.g. one-step RT-PCR versus two-step RT-PCR), and the method employed for subtyping (e.g. nested PCR, blot hybridization with specific DNA probes, restriction site-specific PCR, sequence analysis, etc.).8 To avoid false positive results due to non-specific amplification, it is important to target regions of the genome that are specific to dengue and not conserved
among flavivirus or other related viruses. False-positive results may also occur as a result of contamination by amplicons from previous amplifications. This can be prevented by physical separation of different steps of the procedure and by adhering to stringent protocols for decontamination.43

2.11.2 Isothermal amplification methods

The NASBA (nucleic acid sequence based amplification) assay is an isothermal RNA-specific amplification assay that does not require thermal cycling instrumentation. The initial stage is a reverse transcription in which the single-stranded RNA target is copied into a double-stranded DNA molecule that serves as a template for RNA transcription. Detection of the amplified RNA is accomplished either by electrochemiluminescence or in real-time with fluorescent-labelled molecular beacon probes. NASBA has been adapted to dengue virus detection with sensitivity near that of virus isolation in cell cultures and may be a useful method for studying dengue infections in field studies. Loop mediated amplification methods have also been described but their performance compared to other nucleic acid amplification methods are not known.43

2.11.3 Detection of antigens

Until recently, detection of dengue antigens in acute-phase serum was rare in patients with secondary infections because such patients had pre-existing virus-IgG antibody immunocomplexes. New developments in ELISA and dot blot assays directed to the envelop/membrane (E/M) antigen and the non-structural protein 1 (NS1) demonstrated that high concentrations of these antigens in the form of immune complexes could be detected in patients with both primary and secondary dengue infections up to nine days after the onset of illness.8
The NS1 glycoprotein is produced by all flaviviruses and is secreted from mammalian cells. NS1 produces a very strong humoral response. Many studies have been directed at using the detection of NS1 to make an early diagnosis of dengue virus infection. Commercial kits for the detection of NS1 antigen are now available, though they do not differentiate between dengue serotypes. Their performance and utility are currently being evaluated by laboratories worldwide, including the WHO/TDR/PDVI laboratory network.\(^8\)

Fluorescent antibody, immunoperoxidase and avidin-biotin enzyme assays allow detection of dengue virus antigen in acetone-fixed leucocytes and in snap-frozen or formalin-fixed tissues collected at autopsy.\(^8\)

### 2.11.4 Serological tests

#### 2.11.5 MAC-ELISA

For the IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) total IgM in patients sera is captured by anti-μ chain specific antibodies (specific to human IgM) coated onto a microplate. Dengue-specific antigens, from one to four serotypes (DEN-1, -2, -3, and -4), are bound to the captured anti-dengue IgM antibodies and are detected by monoclonal or polyclonal dengue antibodies directly or indirectly conjugated with an enzyme that will transform a non-coloured substrate into coloured products.\(^8\) The optical density is measured by spectrophotometer. Serum, blood on filter paper and saliva, but not urine, can be used for detection of IgM if samples are taken within the appropriate time frame (five days or more after the onset of fever). Serum specimens may be tested at a single dilution or at multiple dilutions. Most of the antigens used for this assay are derived from the dengue virus envelope protein (usually virus-infected cell culture supernatants or suckling mouse brain preparations). MAC-ELISA has good sensitivity and specificity but only when used five or more days after the onset
of fever. Different commercial kits (ELISA or rapid tests) are available but have variable sensitivity and specificity.\textsuperscript{8} A WHO/TDR/PDVI laboratory network recently evaluated selected commercial ELISAs and first-generation rapid diagnostic tests, finding that ELISAs generally performed better than rapid tests. Cross-reactivity with other circulating flaviviruses such as Japanese encephalitis, St Louis encephalitis and yellow fever, does not seem to be a problem but some false positives were obtained in sera from patients with malaria, leptospirosis and past dengue infection.\textsuperscript{8} These limitations have to be taken into account when using the tests in regions where these pathogens co-circulate. It is recommended that tests be evaluated against a panel of sera from relevant diseases in a particular region before being released to the market. It is not possible to use IgM assays to identify dengue serotypes as these antibodies are broadly cross-reactive even following primary infections. Recently, some authors have described MAC-ELISA that could allow serotype determination but further evaluations are required.\textsuperscript{8,17,41}

2.11.6 IgG ELISA

The IgG ELISA is used for the detection of recent or past dengue infections (if paired sera are collected within the correct time frame). This assay uses the same antigens as the MAC-ELISA. The use of E/M-specific capture IgG ELISA (GAC) allows detection of IgG antibodies over a period of 10 months after the infection. IgG antibodies are lifelong as measured by E/M antigen-coated indirect IgG ELISA, but a fourfold or greater increase in IgG antibodies in acute and convalescent paired sera can be used to document recent infections. Test results correlate well with the haemagglutination-inhibition test. An ELISA inhibition method (EIM) to detect IgG dengue antibodies is also used for the serological diagnosis and surveillance of dengue cases. This system is based in the competition for the antigen sites by IgG dengue antibodies in the sample and the conjugated human IgG anti-dengue.\textsuperscript{8}
This method can be used to detect IgG antibodies in serum or plasma and filter-paper stored blood samples and permits identification of a case as a primary or secondary dengue infection. In general, IgG ELISA lacks specificity within the flavivirus sero-complex groups. Following viral infections, newly produced antibodies are less avid than antibodies produced months or years after infection.

Antibody avidity is used in a few laboratories to discriminate primary and secondary dengue infections. Such tests are not in wide use and are not available commercially.8,11,16

2.11.7 Immunoglobulin M (IgM)/Immunoglobulin G (IgG) ratio
A dengue virus E/M protein-specific IgM/IgG ratio can be used to distinguish primary from secondary dengue virus infections. IgM capture and IgG capture ELISAs are the most common assays for this purpose. In some laboratories, dengue infection is defined as primary if the IgM/IgG OD ratio is greater than 1.2 (using patient's sera at 1/100 dilution) or 1.4 (using patient's sera at 1/20 dilutions). The infection is secondary if the ratio is less than 1.2 or 1.4. This algorithm has also been adopted by some commercial vendors. However, ratios may vary between laboratories, thus indicating the need for better standardization of test performance.4

Immunoglobulin A
Positive detection for serum anti-dengue IgA as measured by anti-dengue virus IgA capture ELISA (AAC-ELISA) often occurs one day after that for IgM. The IgA titer peaks around day 8 after onset of fever and decreases rapidly until it is undetectable by day 40. No differences in IgA titers were found by authors between patients with primary or secondary infections. Even though IgA values are generally lower than IgM, both in serum and saliva, the two methods
could be performed together to help in interpreting dengue serology. This approach is not used very often and requires additional evaluation.\textsuperscript{43}

2.11.8 Haemagglutination-inhibition test

The haemagglutination-inhibition (HI) test is based on the ability of dengue antigens to agglutinate red blood cells (RBC) of ganders or trypsinized human O RBC. Anti-dengue antibodies in sera can inhibit this agglutination and the potency of this inhibition is measured in an HI test. Serum samples are treated with acetone or kaolin to remove non-specific inhibitors of haemagglutination, and then adsorbed with gander or trypsinized type O human RBC to remove non-specific agglutinins.\textsuperscript{1,18} Each batch of antigens and RBC is optimized. PH optimum of each dengue haemagglutinin requires the use of multiple different pH buffers for each serotype. Optimally the HI test requires paired sera obtained upon hospital admission (acute) and discharge (convalescent) or paired sera with an interval of more than seven days. The assay does not discriminate between infections by closely related flaviviruses (e.g. between dengue virus and Japanese encephalitis virus or West Nile virus) nor between immunoglobulin isotypes. The response to a primary infection is characterized by the low level of antibodies in the acute-phase serum drawn before day 5 and a slow elevation of HI antibody titters thereafter. During secondary dengue infections HI antibody titers rise rapidly, usually exceeding 1:1280. Values below this are generally observed in convalescent sera from patients with primary responses.\textsuperscript{8}

2.12 Haematological tests

Platelets and haematocrit values are commonly measured during the acute stages of dengue infection. These should be performed carefully using standardized protocols, reagents and equipment. A drop of the platelet count below 100 000 per $\mu$L may be observed in dengue fever
but it is a constant feature of dengue haemorrhagic fever. Thrombocytopenia is usually observed in the period between day 3 and day 8 following the onset of illness.\textsuperscript{45}

Haemoconcentration, as estimated by an increase in haematocrit of 20\% or more compared with convalescent values, is suggestive of hypovolemia due to vascular permeability and plasma. Other laboratory tests to be carried out for patients with possible dengue are:

- Complete blood count (CBC)
- Metabolic panel
- Serum protein and albumin levels
- Liver panel
- Disseminated intravascular coagulation (DIC) panel

Characteristic findings in dengue fever are as includes:

- Thrombocytopenia (platelet count < 100 \times 10^9/L)
- Leukopenia
- Mild to moderate elevation of aspartate aminotransferase and alanine aminotransferase values
- In patients with dengue haemorrhagic fever, the following may be present:
  - Increased haematocrit level secondary to plasma extravasation and/or third-space fluid loss
  - Hypoproteinaemia
  - Prolonged prothrombin time
  - Prolonged activated partial thromboplastin time
  - Decreased fibrinogen
  - Increased amount of fibrin split products
Guaiac testing for occult blood in the stool should be performed on all patients in whom dengue virus infection is suspected. Urinalysis identifies hematuria.

Imaging studies

- Chest radiography
- Head computed tomography (CT) scanning without contrast: To detect intracranial bleeding or cerebral oedema from dengue haemorrhagic fever.
- Ultrasonography: To detect fluid in the chest and abdominal cavities, pericardial effusion, and a thickened gallbladder wall, in dengue haemorrhagic fever.\textsuperscript{45}

2.13 Treatment/ Prevention

There is no specific treatment for dengue fever. For severe dengue medical care by physicians and nurses experienced with the effects and progression of the disease can save lives decreasing mortality rates from more than 20\% to less than 1\%. Maintenance of the patient’s body fluid volume is critical to severe dengue care.\textsuperscript{43}

2.13.1 Immunization

There is no vaccine to protect against dengue. However, major progress has been made in developing vaccine against dengue and severe dengue. Three tetravalent live-attenuated vaccines are under development in phase two and phase three clinical trials, and three other vaccine candidates (based on subunit, DNA and purified inactivated virus platforms) are at earlier stages of clinical development.\textsuperscript{43}

2.13.2 Prevention and control

The only method to control or prevent transmission of dengue virus is to combat vector mosquitos through the following ways:
- Preventing mosquitos from accessing egg-laying habitat by environmental management and modification.
- Disposing of solid waste properly and removing artificial man-made habitats.
- Covering emptying and cleaning of domestic water storage containers.
- Use of personal household protection such as window screens, coil and vaporizers
- Improving community participation and mobilization for sustained vector control measures.
- Active monitoring and surveillance of vectors should be carried out to determine effectiveness of control interventions.
- Applying insecticides as space spraying during outbreaks as one of the emergency vector-control measures.43

2.13.3 Complications

Virus genome (genetic material) contains about 11,000 nucleotide bases, which code for the three different types of protein molecules (C, prM and E) that form the virus particle and seven other types of protein molecules (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5) that are only found in infected host cells and are required for replication of the virus. The main arthropod vector for the transmission of the DENVs is *Aedes aegypti* and *Aedes albopictus* which are now known to be extensively spread in both tropics and subtropics (41). Infection may be asymptomatic or patients may present with dengue fever (DF), dengue haemorrhagic fever (DHF), or dengue shock syndrome (DSS).46
CHAPTER THREE

METHODOLOGY

3.1 Study Area

Karu LGA was the area of interest selected based on the incidence of dengue haemorrhagic fever (VHF) that occurred in the area in April, 2014. The case patient was studying at Bingham University situated at Karu LGA, who later died after the confirmation of the diagnosis at Lagos University Teaching Hospital. It is believed that the infection was contracted at school considering the incubation period of the virus which is 21 days hence the selection of the study area Karu, Nasarawa State. Nassarawa State is divided into three senatorial districts (South, North, and West), it consists of 13 local governments; Karu, Keffi, Nasarawa Egon, Wamba, Keana, Kokona, Toto, Obi, Doma, Akwanga, Awe, Nasarawa and Lafia as the capital. Karu is a local government area in Nasarawa State. It has eleven wards (Asokodape, Bagagi, Gitata, Gurku Kabusu, Karshi I, Karshi ii, Karu, Keffin Shanu, Panda Kare, Tattare and Uke). Nasarawa State is in north central Nigeria created in October 1996 by the Abacha government. It is bounded in the north by Kaduna State, in the west by Abuja- Federal Capital Territory, in the south by Kogi and Benue States and in the East by Taraba and Plateau States. Its Coordinate are: 8°32’N 8°18’E, with a total area of 27,117km² (10,470 sq mi), a total population of 2,040,097, and has a density of 75/km² (1902 mi). The State has Agriculture as the mainstay of its economy with the production of variety of cash crops throughout the year. It has Solid minerals such as salt and bauxite. Nasarawa state lies within guinea savannah region and has tropical climate with moderate rainfall. It is made up of plain lands and hills measuring up to 300ft above the sea level at some points. Other factors that favours dengue vectors breeding sites due to presence of open gutter and lack of proper drainages and also rapid growth and urbanization due to its proximity to
Federal capital territory with very little infrastructural development such as good urban planning, proper drainage.

Figure 1: Map of Nigeria highlighting Nasarawa State Nigeria in Yellow
3.2 Study Design
A descriptive cross sectional study was carried out from December 2015 to March, 2016.

3.3 Study Population
Apparently healthy individuals that were residing in Karu L.G.A. Nasarawa State, Nigeria.

3.3.1 Selection Criteria
All individuals of both sexes between the of age five years (5) to seventy (70) years that are resident in Karu LGA for at least six months prior to the study.

3.4 Sample Size Determination
The Leslie and Kish formula,\(^47\) was used in estimating the sample size for cross-sectional survey

\[ N = \frac{z^2 pq}{d^2} \]

Where:

- \( n \) = minimum sample size
- \( Z_{\alpha} \) = the standard normal deviate at 5% significance level; 1.96
- \( p \) = estimated prevalence of dengue infection in Nigeria 0.36%. \(^1\)
- \( q \) = 1 - 0.36 = 0.64
- \( d \) = level of precision (allowable error) = 5%

Therefore, \( n_0 = \frac{(1.96)^2 \times 0.36 \times 0.64}{(0.05)^2} \)

Sample size is = 354
3.5 Sampling Technique

Multi-stage sampling technique was used.

Stage 1: List of all the 11 wards in the LGAs (Asokodape, Bagaji Agada, Gitata, Gurku Kabusu, Karshi I, Karshi II, Karu, Keffin Shanu, Panda Kare, Tattara, Vuke) was obtained, and by balloting ten (10) were selected leaving only Karshi II which was used for the pretesting of questionnaires.

Stage 2: List of all the 39 settlements in the wards was made and using simple random technique 14 settlements selected. The fourteen settlements have approximately the same population site

Stage 3: Random sampling technique was used to select minimum of 25 participants per settlement depending on the number of population per settlement till samples size was achieved, from the study population who were eligible and consented to partake in the survey from which blood samples were collected, were as parent consent were abstained in order to collect blood samples from their children.

3.6 Study Instruments

The instruments used for this study are enumerated below:

1. **Questionnaires**: the questionnaire was a structured one having 7 sections as follows:

   which was validated in Karshi II, the only ward not selected for the study;

   - Section 1: General Information
   - Section 2: Individual characteristics
   - Section 3: Awareness of dengue virus infection
   - Section 4: Sign and symptoms
2. **Sample collection:** Two interviewers who were Community Health Extension Workers, 2 phlebotomists who work at the Laboratory as technicians and 2 local guides were recruited and trained on the protocol for duration of 3 days as research assistants. The pretested and validated questionnaires were administered to the study participants that consented to participate in the study. Five mL of blood was collected from each individual using aseptic technique. Samples were transported to the laboratory using proper packaging and transportation equipment. Samples stored at – 20°C at Maitama Measles/Rubella and Yellow fever Laboratory until ready for use.

### 3.7 Sample Processing

#### 3.7.1 Principles of ELISA test using ELISA kit

IgG/IgM ELISA kit manufactured by AccuDiag™ Dengue IgG ELISA Kit, Cat # 8116-35. Lot Number DA1290, DA1289, DA1289, DA1290 for primary testing and determination of acute and convalescent dengue infection. The Dengue IgG ELISA Test enzyme linked immunosorbent assay (ELISA) is for semi-quantitative identification of anti-bodies to dengue in human serum. The Dengue IgG ELISA kit is to be performed in a clinical laboratory setting only.48

#### 3.7.2 Antibody Detection

The dengue IgG detection involves three incubation stages bases on the kit instruction. Before the first incubation stage the micro wells are coated with purified dengue virus antigen from Vero cell culture type 1-4 culture. The patient sera are added, and if there are any anti-bodies
present they will bind to the wells during the first incubation. The wells are then washed of any test samples and enzymes conjugate are then added and incubated and washed, then chromogen is added with the presence of enzyme conjugate and peroxidase causing the consumption of peroxide. The chromogen changes colour to blue. After the addition of stop solution the blue colour turns yellow which ends the reaction. Results are read using ELISA reader or it can be read visually.  

3.7.3 ELISA Reader: Is set at zero reader on air. It is set for biochromatic readings on 450/620-650nm using the automated washing option B (blank) which is set to use the background option.

3.7.4 Test interpretation: Samples interpreted as non-reactive are from 0.0-0.3 OD units, or zero colour) indicating that antibodies are not present in the sample. Samples interpreted as strongly reactive (>1.0 OD) indicate the presence of specific antibody.

3.8 Data Management

All data collected was standardized to facilitate data comparison and analysis and double data entry was done to ensure accuracy.

3.8.1 Data Entry

To ensure data quality, all filled questionnaires were checked for errors. The data was cleaned to check for missing and logically impossible findings and records. Frequency tables, bar charts and histograms were used to check for unlikely values and outliers. Resort was made to original questionnaires and records verification.

3.8.2 Statistical analyses

Epi-info version 7.1.4.0 was used to analyse data and also Microsoft excel. Univariate, bivariate, multivariate and regression analysis was performed to compare proportions within and among
groups for statistical significance and also to consider multi-factorial causes of effect at a P value < 0.05.

3.8.3 Measurement of variables

**Exposure variables:** Exposure variables obtained from the study were; age, sex, occupation, educational level, risk factors, and proper environmental sanitation, waste disposal and knowledge of dengue.

**Outcome variable:** The outcome variable was the presence of dengue virus anti-bodies (IgG) in the collected blood sample (serum).

3.9 Ethical Considerations

Ethical approval to conduct this research was obtained from the Nasarawa State Ministry of Health ethical committee as well as written and informed consent from the participants. For children consent was obtained from their parents and guardians before administration of questionnaire and sample collection. Confidentiality, none harmfulness of the study was assured to the study participants. An advocacy visit to the LGA and community leaders was done to gain entry into the community.

3.10 Limitations

The cross reactivity among the flavivirus family and inability to conduct the vector components of the study to find out the implicating vector was considered to be the limitation of this survey.
CHAPTER FOUR

RESULT

Table 4.1: Socio-demographic characteristics of the respondents

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Frequency</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age-group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-14</td>
<td>138</td>
<td>39.0</td>
</tr>
<tr>
<td>15-29</td>
<td>112</td>
<td>31.6</td>
</tr>
<tr>
<td>30-44</td>
<td>64</td>
<td>18.1</td>
</tr>
<tr>
<td>&gt;45</td>
<td>40</td>
<td>11.3</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>186</td>
<td>52.5</td>
</tr>
<tr>
<td>Female</td>
<td>168</td>
<td>47.4</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Business</td>
<td>80</td>
<td>22.6</td>
</tr>
<tr>
<td>Civil servant</td>
<td>18</td>
<td>5.1</td>
</tr>
<tr>
<td>Farmer</td>
<td>43</td>
<td>12.1</td>
</tr>
<tr>
<td>Private sector</td>
<td>11</td>
<td>3.1</td>
</tr>
<tr>
<td>Unemployed/Student</td>
<td>202</td>
<td>57.1</td>
</tr>
<tr>
<td><strong>Level of education</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>130</td>
<td>36.7</td>
</tr>
<tr>
<td>Secondary</td>
<td>123</td>
<td>34.8</td>
</tr>
<tr>
<td>Tertiary</td>
<td>33</td>
<td>9.3</td>
</tr>
<tr>
<td>Qur'anic</td>
<td>47</td>
<td>13.8</td>
</tr>
<tr>
<td>Adult/informal education</td>
<td>19</td>
<td>5.4</td>
</tr>
</tbody>
</table>

Table 4.1 above shows the demographic characteristics of the respondents. The age of the participants ranged from 5 to > 45 years old and the median age was 23 years. Majority of the participants were aged 5-29 years old- 250 (70.7%). Out of the total number 168 (47.5%) were females and 186 (52.5%) were males. More than half (57.1%) of the participants were Unemployed/students. Those with primary school level of education have the highest participation 130 (36.7%).
Table 4.2: Sero-prevalence of dengue antibody by socio-demographic factors among the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number tested</th>
<th>Number positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>186</td>
<td>9 (4.8)</td>
</tr>
<tr>
<td>Female</td>
<td>168</td>
<td>8 (4.8)</td>
</tr>
<tr>
<td><strong>Age-group (in years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-14</td>
<td>138</td>
<td>3 (2.2)</td>
</tr>
<tr>
<td>15-29</td>
<td>112</td>
<td>5 (4.6)</td>
</tr>
<tr>
<td>30-44</td>
<td>64</td>
<td>2 (3.1)</td>
</tr>
<tr>
<td>45 Above</td>
<td>31</td>
<td>7 (22.5)</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farmer</td>
<td>43</td>
<td>8 (18.6)</td>
</tr>
<tr>
<td>Civil Servant</td>
<td>18</td>
<td>2 (11.1)</td>
</tr>
<tr>
<td>Private sector</td>
<td>11</td>
<td>1 (9.0)</td>
</tr>
<tr>
<td>Business</td>
<td>80</td>
<td>13 (3.8)</td>
</tr>
<tr>
<td>Unemployed/Students</td>
<td>202</td>
<td>3 (1.5)</td>
</tr>
<tr>
<td><strong>Level of education</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>130</td>
<td>3 (2.3)</td>
</tr>
<tr>
<td>Secondary</td>
<td>123</td>
<td>5 (4.1)</td>
</tr>
<tr>
<td>Tertiary</td>
<td>33</td>
<td>2 (6.1)</td>
</tr>
<tr>
<td>Quranic</td>
<td>49</td>
<td>2 (4.1)</td>
</tr>
<tr>
<td>Informal education</td>
<td>19</td>
<td>5 (26.3)</td>
</tr>
</tbody>
</table>

Table 4.2 above shows 17 (4.8%) out of 354 participants tested positive to Dengue IgG antibodies. Both male and females had a prevalence of 4.8%. Age group 45> has the highest seroprevalence with 22.5% while the least seroprevalence was in agegroup 5-14 years 2.2 %. Occupations of the participant with the highest seroprevalence were farmers with 18.6% while those with informal education had the highest seroprevalence.
Figure 4.2: Frequency and percentage of dengue IgG results among the study population in Karu L.G.A. Nasarawa.
Those with informal education that are farmers tend to have the highest number of dengue IgG seroprevalence with 3(37.5%). While those are have Qur’anic and secondary education that are farmers have the second highest sero-positivity with 2(1.7%) and 2(22.2%) respectively. Primary school children that engage in farming have 1(16.7%). Civil servant with tertiary level of occupation 2(15.4%) were sero-positive.
Table 4.3: Risk factors and dengue sero positives among study participants in Karu LGA Nasarawa State

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Number</th>
<th>IgG positive</th>
<th>%</th>
<th>Odds ratio</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Engaged in farming activities</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes:</td>
<td>126</td>
<td>9</td>
<td>7.1</td>
<td>1.88 (0.70 - 5.0)</td>
<td>0.30</td>
</tr>
<tr>
<td>No:</td>
<td>211</td>
<td>8</td>
<td>3.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keeping waste bin in the house</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes:</td>
<td>231</td>
<td>7</td>
<td>3.03</td>
<td>0.32 (0.12 - 0.86)</td>
<td>0.03</td>
</tr>
<tr>
<td>No:</td>
<td>106</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sleeping outside</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes:</td>
<td>171</td>
<td>8</td>
<td>4.7</td>
<td>0.86 (0.32 - 2.29)</td>
<td>0.96</td>
</tr>
<tr>
<td>No:</td>
<td>166</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of street gutters and drainages</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes:</td>
<td>117</td>
<td>2</td>
<td>1.7</td>
<td>0.25 (0.05 - 1.11)</td>
<td>0.09</td>
</tr>
<tr>
<td>No:</td>
<td>220</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keeping water in an open container</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes:</td>
<td>82</td>
<td>2</td>
<td>2.5</td>
<td>0.41 (0.09 - 1.85)</td>
<td>0.37</td>
</tr>
<tr>
<td>No:</td>
<td>255</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of window nets</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes:</td>
<td>141</td>
<td>3</td>
<td>2.1</td>
<td>0.29 (0.08 - 1.05)</td>
<td>0.08</td>
</tr>
<tr>
<td>No:</td>
<td>196</td>
<td>14</td>
<td>7.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of mosquito net at the night</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes:</td>
<td>125</td>
<td>10</td>
<td>8.0</td>
<td>2.42 (0.89 - 6.52)</td>
<td>0.12</td>
</tr>
<tr>
<td>No:</td>
<td>212</td>
<td>7</td>
<td>3.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of vegetation cover around the house</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes:</td>
<td>56</td>
<td>0</td>
<td>0</td>
<td>undefined</td>
<td></td>
</tr>
<tr>
<td>No:</td>
<td>281</td>
<td>17</td>
<td>6.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you spray your environment daily</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes:</td>
<td>61</td>
<td>0</td>
<td>0</td>
<td>Undefined</td>
<td></td>
</tr>
<tr>
<td>No:</td>
<td>276</td>
<td>17</td>
<td>6.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>How often do you clean the gutters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Biweekly</td>
<td>13</td>
<td>1</td>
<td>7.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Daily</td>
<td>87</td>
<td>5</td>
<td>5.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Never</td>
<td>16</td>
<td>11</td>
<td>68.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Risk factors to dengue infection showed only keeping waste bin around the house to be statistically significant with (OR 0.32, CI (0.12-.86)
CHAPTER FIVE

DISCUSSION

This study found a sero-prevalence of 17 (4.8%) to Anti Dengue Virus IgG anti-bodies indicating previous infection to dengue virus. This study has proven that dengue virus is circulating in this community and is being under reported or not reported at all. A study conducted in Maiduguri found a sero-prevalence of 0.6% in 2009 compared to our study, the prevalence is much higher. Also this indicated that the prevalence of the disease is increasing as it was reported by several other studies.\textsuperscript{3,7,43}

This study considered the prevalence to be high because the study assessed only one LGA out of 13 in the state and among apparently healthy asymptomatic individuals in the community as compared to other studies conducted among febrile patients across the four geo-political zones of Nigeria with prevalence of 0.67% in 2009, 0.5% in 2011 and 10.1 % in 2013, all conducted in Maiduguri.\textsuperscript{7,8} This suggested that if the study were to be spread across all the LGAs there is likelihood of getting much higher prevalence of dengue anti-bodies especially among symptomatic patients with fever and other risk factors of the disease.

Our survey determined the sero-prevalence of DENV antibodies to be higher in age group > 45 years and above with (22.5%) prevalence as shown in Table 3. While our study found higher prevalence at a much higher agegroup anoher studies with a dissimilar findings conducted in 2006 found sero-prevalence of dengue antibodies to be higher in very young children ages 4-6 years old with a steady increase at age 4 and 100% at age 16 with 91%, 75% and 100% respectively.\textsuperscript{49}
In another survey conducted by Idris et al in 2013 documented high prevalence of dengue antibodies among age-group 30-39 year in Maiduguri.\textsuperscript{19}

This study found sero-prevalence to be the same in both males and females with (4.8\%) each. In another study in Singapore that is quite dissimilar from this study reported dengue IgG seroprevalence to be significantly higher in males than females with 1.21 times more likely for males to be exposed to dengue than females.\textsuperscript{47} The slight difference found in the study could be attributed to the fact that males are more involved in outdoor activities than their female counter parts, and dengue vectors (\textit{Aedes aegypti} and \textit{Aedes Albopictus}) are day biting mosquitos and males also tends to be more involved in outdoor activities than their female counter parts.\textsuperscript{50} Another study conducted in Ibadan Nigeria similar with our study findings found an equal female to male ratio in terms of disease seroprevalence.\textsuperscript{7} Brunkard’s study was quite dissimilar with higher seroprevalence of dengue among females than males.

Age-group 20-29 years old and > 50 were found to have the highest number of sero-positivity of (7.2\%) and (29.4\%) respectively this is consistent with studies conducted by Baba and Talle,\textsuperscript{51} which found an increase in vector infection with age and it could be attributed to nature of female mosquito developmental stage where at an immature stage they are found in water filled habitat mostly in artificial containers closely associated with human dwellings and often indoors and spend most of their time around the house people rather than mosquitos can move the virus within and between communities, therefore, the most active group of the population tends to be at risk of mosquito bite and spread the disease.\textsuperscript{8}

Occupation of the participants’ assessed showed farmers had the highest sero-positivity of (18.6\%).\textsuperscript{46} Those that engaged in farming activities had higher sero-positivity of (18.6\%)
compared to those that did not engage in farming activities with reason this could be attributed
to the duration of time the farmers spend outside the house in the farm. Other risk factors
assessed included sleeping outside the house, keeping water in an open container, presence of
gutters around the house was found to have no effect on the sero-positivity of the study
participant even though other studies by Gunaseka found positive effect of keeping water in an
open container. Also based on the fact this interview was questionnaire based it is possible that
the participants may provide socially desirable responses regarding practices that could affect
lack of association even if there is any. The Use of mosquito nets during the night was found not
to be protective as dengue mosquitos bite during the day.

But participant with no window net 14 (7.1%) were positive while does with window nets only 3
(2.1%) were positive this was found to have significant effect to dengue infection which is
consistent with a study by Gunaseka.

Dengue disease inflicts a significant health and economic and social burden on the population of
endemic areas and serves as a source of concern since the asymptomatic individuals with the
virus can also transmit the infection to mosquitoes during blood meal and vis-versa. Hence;
calls for more studies to further investigate and determine the burden of the disease especially in
the areas where sporadic cases has been reported to avoid salient transmission of the infection
within communities is required.
CHAPTER SIX
CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study investigated the presence of dengue virus circulation among the study population. The sero-prevalence of dengue anti-body was found to be high in Karu local government area of Nasarawa State. This has indicated that dengue virus is circulating within the community contributing to a significant number of febrile cases. Agegroup 45 years and above were found to have highest sero-prevalence of dengue virus antibody. Farming and keeping waste around the compound was found to be a risk factor to dengue virus infection.

6.2 Recommendations

From the result obtained from this study, a sero prevalence of 4.8% among healthy participant in Karu LGA and dengue diagnosis is not routinely done in the hospitals nor is there a surveillance activity for dengue diseases in the state and the country at large; it is highly recommended that the both the Federal and State government to:

1. Local. State and Federal government to establish a surveillance system for dengue virus and possibly other viral haemorrhagic diseases in the country.

2. Local government and health facilities to create awareness at the health facilities in the LGA as well as at the community level in the form of health educate/promotion to share and pass information on dengue vectors, its symptoms, method of transmission and prevention.

3. High index of suspicion to be raised among the health care workers in the hospitals to avoid missing any case of related to viral haemorrhagic fever.
4. Diagnosis of dengue to be included as part of routine investigations and also as a differential diagnosis for febrile illnesses in the general hospitals across the country to be able to distinguish and identify the appropriate aetiology of a disease.

5. Nasarawa state environmental protection board to ensure proper disposal of waste and provision of drainages in both villages and sub-urban cities in the state.

6. Vector control through spraying of the environment by the government at the LGA, State and Federal level.
REFERENCES


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34. Pinto LMO, Oliveira SA, Braga ELA, Nogueira R, Kubelka CF. Increased Pro-inflammatory Cytokines ( TNF- a and IL-6 ) and in Brazilian Patients during Exanthematic Dengue Fever. 1999;94(3):387–94.


47. Gerritsen A. Sample size calculation in Cross Sectional Studies. 2015 Epi Result Article


APPENDICES

Appendix 1

SERO SURVEY ON PREVALENCE OF DENGUE ANTI-BODY AMONG RESIDENTS IN KARU LOCAL GOVERNMENT AREA, NASARAWA STATE, NIGERIA

INTERVIEWER-ADMINISTERED QUESTIONNAIRE

Introduction: This questionnaire is strictly for the purpose of conducting investigation on the above mentioned topic by me as a student of Ahmadu Bello University Zaria, Department of Community Medicine for the award of Master Degree in Public Health. It is education based, strictly consensual and all information will be treated with confidentiality.

Dengue is an acute systemic viral infection caused by four serotypes of the dengue virus (DENV). It is transmitted through the bite of an infected mosquito mainly A. aegypti and A. albopictus which transmit it to humans and found mainly in tropical and sub-tropical regions of the world. Recently transmission has increased primarily in urban and semi-urban areas and has become a major international public health concern. Dengue fever have similar symptoms with malaria most often it’s diagnosed as malaria, mixed infection occur and this puts a person at risk of viral hemorrhagic fever.

<table>
<thead>
<tr>
<th>1. General</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 Date of Survey (dd/mm/yy)</td>
</tr>
<tr>
<td>1.2 Name of Interviewer</td>
</tr>
<tr>
<td>1.3 Name of community</td>
</tr>
<tr>
<td>1.4 State &amp; LGA</td>
</tr>
<tr>
<td>1.5 Phone number</td>
</tr>
<tr>
<td>1.6 Location (GIS Coordinates)</td>
</tr>
</tbody>
</table>
2. Individual Characteristics

<table>
<thead>
<tr>
<th>2.1</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2</td>
<td>Sex</td>
</tr>
<tr>
<td></td>
<td>Male</td>
</tr>
<tr>
<td>2.3</td>
<td>Marital status</td>
</tr>
<tr>
<td></td>
<td>Married</td>
</tr>
<tr>
<td>2.4</td>
<td>Education</td>
</tr>
<tr>
<td></td>
<td>Informal/Adult education</td>
</tr>
<tr>
<td></td>
<td>Primary</td>
</tr>
<tr>
<td>2.5</td>
<td>Occupation</td>
</tr>
<tr>
<td></td>
<td>1. Private sector</td>
</tr>
<tr>
<td></td>
<td>3. Other specify</td>
</tr>
<tr>
<td>2.6</td>
<td>Tribe</td>
</tr>
<tr>
<td></td>
<td>1 Hausa</td>
</tr>
</tbody>
</table>

3. Awareness of Dengue Virus Infection

<table>
<thead>
<tr>
<th>3.1</th>
<th>Are you aware of dengue infection?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. Yes</td>
</tr>
<tr>
<td>3.2</td>
<td>Are you aware of its signs/symptoms</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>3.3</td>
<td>When was the last time you had fever</td>
</tr>
<tr>
<td></td>
<td>1. One week ago</td>
</tr>
<tr>
<td></td>
<td>3. One month ago</td>
</tr>
<tr>
<td></td>
<td>5. Three months ago</td>
</tr>
<tr>
<td>3.4</td>
<td>Have received any treatment?</td>
</tr>
<tr>
<td>-----</td>
<td>-----------------------------</td>
</tr>
</tbody>
</table>

4. **Sign and Symptoms**

<table>
<thead>
<tr>
<th>4.1</th>
<th>Have you experienced the following symptom in the past three months?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2</td>
<td>Headache</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>4.3</td>
<td>Fever</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>4.4</td>
<td>Nausea/Vomiting</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>4.5</td>
<td>Weakness</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>4.6</td>
<td>Sore throat</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>4.7</td>
<td>Weakness</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>4.8</td>
<td>Rash</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>4.10</td>
<td>Anorexia</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>4.11</td>
<td>Altered taste sensation</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

5. **Environmental factors**

<table>
<thead>
<tr>
<th>5.1</th>
<th>Do you leave your waste bin around the house?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.2</td>
<td>How often do you discard your waste?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>5.3</td>
<td>Do you store water in an open container?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>5.4</td>
<td>Do you sleep under mosquito nets?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>5.5</td>
<td>Do you have open gutters near the house?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>5.6</td>
<td>Is your house near or surrounded by a bush?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>5.7</td>
<td>Do you spray your environment daily?</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

6. **Disease Management**
6.1 Have you received yellow fever Vaccinations?  | Yes | No
---|---|---
6.2 How often do you have fever?  | Quite often | Once in a month
| Once in two months | Once in three months
6.3a Have you visited the hospital?  | 1. Yes | If yes was a test conducted? | Name medication
6.3b If no, have you taken any medication  | Yes | No

7. Personnel & Sanitation

<table>
<thead>
<tr>
<th>7.1</th>
<th>How often do you clean your house and surroundings?</th>
<th>1. Always</th>
<th>2. Sometimes</th>
<th>3. Never</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.4</td>
<td>Do you use window nets in your house?</td>
<td>1. Always</td>
<td>2. Sometimes</td>
<td>3. Never</td>
</tr>
<tr>
<td>7.1</td>
<td>Have you had any vaccination for yellow fever, or avian influenza in the past?</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>--------------------------------------------------------------------------------</td>
<td>-----</td>
<td>-----</td>
<td></td>
</tr>
</tbody>
</table>

8. Socioeconomic Factors

<table>
<thead>
<tr>
<th>8.1</th>
<th>Do you sleep outside the house during hot season?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>8.2</th>
<th>Do you engage in farming or other related activities?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>
Appendix 2

Field site activities (interview, and sample collection) and sample processing in the laboratory.
Appendix 3

ETHICAL CLEARANCE

SECRET
NASARAWA STATE OF NIGERIA
MINISTRY OF HEALTH

Ministry of Health Headquarters
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Lafia, Nasarawa State
Email: nsMohla@Yahoo.com

Telephone:

S/MOH/843/VOL.1/XX

9th November, 2015

Adama Ahmed Abubakar,
Nigeria Field Epidemiology
Training Program (NFELIP)
50 Haile Selasie Street, Asokoro,
Abuja.

RE: APPLICATION FOR ETHICAL CLEARANCE

Reference to your letter 12th October, 2015 on the above subject matter to conduct study titled “Sero-prevalence of dengue infection in Keffi Local Government Area, Nasarawa State, Nigeria”, I am to convey ministry approval to you to do the study.

2. Accordingly, you are to adhere to the methodology in your proposal as well as request for permission from heads of health facilities you chose the study.

3. You must forward 2 copies of the report at the end of the study our records, please.

Dr Ekom G. Haruna
Director Clinical Services

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