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SEROLOGICAL INDICATION OF ZIKA VIRUS INFECTION IN FEBRILE PATIENTS AT THE GREATER ACCRA REGIONAL HOSPITAL, ACCRA GHANA

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This thesis/dissertation is submitted to the University of Ghana, Legon in partial fulfilment of the requirement for the award of MPHIL Microbiology degree

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DECLARATION

I Godson Aryee Ankrah declare that, this research was carried out by me at the Department of Virology, NMIMR. It was supervised by Professor Theophilus K. Adiku, Department of Medical Microbiology, SBAH and Dr. Joseph H. Kofi Bonney, Department of Virology, NMIMR. Work by other investigators which served as a source of information has been acknowledged in the form of referencing.

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(Co-supervisor)

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DEDICATION

I dedicate this research work to God for his guidiance.

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ACKNOWLEDGEMENT

I am thankful to God for the strength and grace given me to successfully complete this work. It was not always smooth, but the Lord has always been there to help. I want to sincerely acknowledge the support of my supervisor Professor Theophilus K. Adiku, a lecturer at the Department of Medical Microbiology, University of Ghana. I appreciate all his useful suggestions.

I would like to also show my appretiation to Dr. Joseph H. Kofi Bonney, senior research fellow, NMIMR for his motivation and constructive criticisms.

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LIST OF ABBREVIATIONS

CDC	Centre for Disease Control and Prevention
RNA	Ribonucleic acid
IgG	Immunoglobulin G antibodies
IgM	Immunoglobulin M antibodies
RT-PCR	Real time polymerase chain reaction
TBM	3, 3‘, 5, 5‘ tetramethylbenzidin
ANOVA	Analysis of Variance
C	Capsid Protein
E	Envelop Protein
FL	Fusion Loop
HRPO	Horseradish Peroxidase
NIAID	National Institute for Allergy and Infectious Disease
NMIMR	Noguchi Memorial Institute for Medical Research
NS	Non-Structural Protein
OD	Optical Density

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PRNT	Plaque Reduction Neutralization Test
PrM	Precursor Membrane
SL	Stem Loop
UTR	Untranslated Region
M	Membrane Protein
IIFA	Indirect Immunofluorescence Assays (IIFA)
VNT	Virus Neutralization Tests
ELISA	Enzyme Linked Immunosorbance Assay
WHO	World Health Organization
USA	United State of America
MTPase	N-terminal S-adenosyl Methionine Methyltransferase
DC-SIGN	Dendritic Cell-Specific Intercellular adhesion molecule -3- Grabbing Non-integrin
ECDC	European Centre for Disease Prevention and Control
NIAID	Nation Institute of Allergy and Infectious Disease
IFRC	International Federation of Red Cross

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UNDP	United Nations Development Programme
NMIMR	Nugochi Memorial Institute for Medical Research

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ABSTRACT

Background: Zika virus infection shares overlapping signs and symptoms with endemic diseases malaria and typhoid fever. These similarities have led to a high probability of false positive diagnosis leading to the over-diagnosis and over-emphasis of these endemic diseases to the neglect of other febrile illnesses of different aetiological agents. This study sought to serologically detect Zika virus infection in febrile patients in a selected health facility, the Greater Accra regional hospital in Ghana.

Method: Archived human sera obtained from febrile patients and stored under ultra-low freezing conditions (-80°C) at Noguchi Memorial Institute for Medical Research were used. An Enzyme linked immunosorbent assay (ELISA) was used in the laboratory to detect anti-Zika virus antibodies (ZIKV IgM and IgG) in the sera.

Results: Out of 160 sera evaluated, 119 were from females with an average age of 30 years whiles the males were of an average age of 29 years. In all, 30 were found to be positive for anti-Zika virus IgM and 3 for anti-Zika virus IgG. No patient had both anti-Zika virus IgG and IgM. Nineteen (63.3%) of the positives were from female patients and 14 (36.7%) from male patients. The highest proportion of seropositives were recorded among the 21-30 year age group with 8 (26.7%) anti-Zika virus IgM and 2 (66.7%) anti-Zika virus IgG. Monthly distribution of anti-Zika virus IgM peaked in March 7 (24.2%) and May 9 (30%) and decreased in September 1 (3.3%). Anti-Zika virus IgG were detected only in January 2 (66.7%) and March 1 (33.3%). Sera from patients who reside at Lapaz and Amasaman recorded the highest seropositivity of 12.1% each. All seropositive febrile patients developed at least one symptom consistent with Zika virus infection: 33 (100%) fever, 25 (76%) muscle pain, 24 (73%) joint pain, and conjunctivitis 2(1.1%). In addition to this, 28 (85%) loss of appetite, 14 (75%) rapid respiration and chest pain 15 (42%) were reported by seropositive febrile patients. Bivariate logistic regression analysis revealed an association between Zika virus seropositivity and chest pain, conjunctivitis, muscle pain and joint pain. A recent hearing loss was the complication detected.

Conclusion: Data from the study indicates exposure to Zika virus which suggests the possible circulation of the virus among febrile patients in Ghana. This study therefore underscores the importance for sero-epidemiological surveillance for timely identification of Zika virus disease to prevent outbreaks.

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CHAPTER ONE

INTRODUCTION

1.1 Background

Fever is a common medical symptom of many diseases (O'Neill, 1994). The sudden onset of fever may herald a serious life-threatening disease or a mild infection which could be of a virus, bacteria and/ or parasite (Wright *et al.*, 1981). In Ghana, febrile illnesses account for about 30% and 75% (Farrar, 2011) of healthcare visit by children and adults (Kiekkas *et al.*, 2013) respectively. However, these febrile illnesses, when presented at healthcare facilities are mostly treated for endemic diseases such as malaria and typhoid fever. This is because, case management of these endemic diseases in most healthcare facilities in Ghana are often by clinical judgement based on signs and symptoms (Chandler *et al.*, 2008). However, clinical judgement based on signs and symptoms is of low specificity (52-61%) due to the overlapping clinical features of Zika virus infection with malaria and typhoid fever (Chandramohan *et al.*, 2002).

This, according to Chandramohan *et al.*, (2002) and Nankabirwa *et al.*, (2009) has led to a high probability of false positive diagnosis which inturn has contributed to the over-diagnosis and over-emphasis of these endemic diseases to the neglect of other febrile illnesses of different aetiological agents. A study conducted in Nigeria showed that 83% of children presented to the hospital with febrile illnesses were treated for malaria with artemisinin-based combination therapy (ACT) before microscopy results were shown to be negative (Oladosu and Oyibo, 2013). Similarly, in a study conducted in Tanzania, 870 hospital admissions showed that malaria was the clinical diagnosis given to 528 (61%) of the patients. However, only 14 (2%) were found to have malaria as the cause of the fever

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by laboratory confirmation (Crump *et al.*, 2013). This is not different in Ghana; less than a third of febrile illnesses are diagnosed as malaria before confirmation with laboratory tests (John Snow Incorporated Research and Training Institute, 2013).

Zika virus infection shares overlapping signs and symptoms with these endemic diseases and has been implicated as the cause of some febrile illnesses around the world (Center for Disease Control and Prevention, 2016). In Africa, the first serological evidence of Zika virus infection in febrile patients was reported in 1975 at the University College Hospital, Ibadan in Nigeria (Moore *et al.*, 1975). Although Zika virus infection accounts for about 3 – 11% of febrile illnesses around the world, it remains under-recognized and under-reported in Ghana (Amarasinghe *et al.*, 2011).

Zika virus infection is arthropod borne (Speer & Pierson, 2016). The etiologic agent, Zika virus is among several neglected arboviruses with apparent pathogenicity in humans (Weissenböck *et al.*, 2001). Zika virus was first isolated in Uganda in 1947 from a rhesus macaque monkey and *Aedes africanus* mosquito in 1948 (Duffy *et al.,* 2009). Subsequently, Zika virus was isolated from other species of *Aedes* mosquitoes such as *Aedes luteocephalus, Aedes aegypti, Aedes albopictus, Aedes furcifer and Aedes vittatus. Aedes* spp. of mosquitoes reproduce in water and eggs are extremely hardy (Paixão *et al.,* 2016). Viral reservoirs are monkeys and transmission is through the bite of *Aedes* spp. of mosquitoes, primarlly *Aedes aegypti* or *Aedes albopictus* which have acquired the virus from an infected monkey through a blood meal (Vasilakis & Weaver, 2016; Ferreira-de-Brito *et al.,* 2016). Other means of transmission include sexual intercourse, vertical and blood transfusion (Oehler *et al.,* 2014).

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In Africa, Zika virus infection was first documented in human in Uganda and Tanzania in 1952 (Haddow *et al.*, 2012) and 1954 in Asia, notably India (Smithburn, 1954). Afterwards, Zika virus infection was found in Egypt, Nigeria, India, Pakistan, North Vietnam and Philippines, spreading from Africa to Southeast Asia (Calveta *et al.*, 2016: Lanciotti *et al.*, 2008). Zika virus infection in Asia and Africa never got the deserved attention due to the sporadic nature of the infection coupled with mild short-term fever. Other symptoms of Zika virus infection include pruritic rashes, non-purulent conjunctivitis and arthralgia (Brasil *et al.*, 2016). As a result, no efforts were made to develop drugs and vaccine (Lanciotti *et al.*, 2008).

Since then, Zika virus infection never occurred in humans outside Africa and Asia until 2007, when the largest outbreak was reported in the Yap State of Micronesia (Duffy *et al.*, 2009: Lanciotti *et al.*, 2008). Outbreak was again documented in the French Polynesia with approximately 28,000 cases in 2013 (Musso *et al.*, 2016: Klase *et al.*, 2016). The devastating aspect of Zika virus infection reported during these outbreaks was the ability of the virus to infect the developing fetus causing birth defects such as microcephaly and intracranial calcifications, and causing Guillain-Barré Syndrome in adults (Coley, 2016). Subsequent spread and the congenital anomalies associated with Zika virus infection led the World Health Organization in 2016 to declare Zika virus infection as a worldwide public health emergency (Chan *et al.*, 2016).

Currently there are no treatment and vaccines available. Diagnosis is only possible in the 2 – 7 days of the infection and is based on detection of specific antibodies by IgG and IgM ELISA, indirect immunofluorescence assays (IIFA) and virus neutralization tests (VNT) (Waggoner and Pinsky, 2017). Virus isolation (Kostyuchenko *et al.*, 2016) and detection of

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viral nucleic acid by RT-PCR (Dai *et al.*, 2016) from cerebrospinal fluid, saliva, serum and urine are also used.

1.2 Problem Statement

Currently, the geographical range of Zika virus infection and the number of susceptible individuals are predicted based on the distributions of *Aedes aegypti* and *Aedes albopictus* (Centers for Disease Control and Prevention, 2016). The presence of active vector population of *Aedes* spp. of mosquitoes in Ghana coupled with proof of contact with the virus in our neighbouring countries (Waddell and Greig, 2016), have necessitated the need for us to document the exposure levels in febrile patients (Plourde and Bloch, 2016). Additionally, in sub-Saharan Africa, febrile cases are mainly treated for endemic diseases like malaria and typhoid fever missing out on other possible aetiologies. Furthermore, the mild or asymptomatic nature and the overlapping clinical features with other endemic disease conditions make it probable for typical Zika virus infection to be missed out in diagnosis. This study therefore gives us the opportunity to document the exposure levels to Zika virus in febrile patients.

1.3 Justification

The possibility that adverse clinical conditions can occur underscore the need to reinforce surveillance for Zika virus and in case of an outbreak, establish rigorous clinical monitoring to detect microcephaly, Guillain-Barré Syndrome and any other unusual clinical manifestations. In light of this, it is important to carry out a study to give an indication of

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the levels of exposure and possible infection due to the Zika virus. This study will determine whether suspected febrile cases presented to the Greater Accra regional hospital have had an exposure to Zika virus. Information that will be obtained on this infection such as prevalence and clinical manifestations will be helpful to healthcare policy makers, providers and health promotion activities. It will also justify the reasons to invest resources to carry out detailed studies to look for clinical cases of Zika viral disease at the Greater Accra regional hospital. This effort will enhance the work by health staff in the clinical determination of suspected Zika cases. This research will add to the body of knowledge in Ghana and the world at large.

1.4 Main Objective

To determine Zika virus seroprevalence in febrile patients at the Greater Accra regional hospital, Ghana

1.4.1 Specific Objectives

- To determine the prevalence of anti-Zika virus antibodies in febrile patients

- To determine the clinical and demographic characteristics of sero-positive patients

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CHAPTER TWO

LITERATURE REVIEW

2.1 Structure, Genomic Organisation and Replication

2.1.1 Structural Morphology of Zika Virus

Zika virus is a single-stranded, enveloped icosahedral virus. Genome has a positive-sense RNA (Shi, 2012). Virion is 40–60 nm in diameter and contains 10,794 nucleotide encoding 3,419 amino acids (Kuno & Chang, 2007). Viral RNA has a distinct stretched open reading frame. This open reading frame encodes a polyprotein translated into three structural proteins (precursor membrane, capsid, and envelope) and seven non-structural proteins (5-C-prM-ENS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3) (Figure 2.1). Viral RNA is encapsulated in a C-protein with precursor membrane and envelope attached. The envelop protein facilitates attachment and entry of virus by membrane fusion (Rey *et al.*, 1995; Nybakken *et al.*, 2006). The envelope protein, capsid protein and precursor membrane play no part in viral replication and are only synthesized to package the matured virus (Pang *et al.*, 2001; Alvarez *et al.*, 2006).

The non-structural proteins (5-C-prM-ENS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3) are responsible for viral replication and are often expressed in the cytoplasm of the infected cell (Pang *et al.*, 2001; Alvarez *et al.*, 2006). NS1 is a glycoprotein and possesses two glycosylation sites which are conserved among flaviviruses. NS1 functions in viral infection and elicits immune response (Avirutnan *et al.*, 2010; Schlesinger *et al.*, 1987). NS3 has three enzymatic activities such as 5' RNA-triphosphatase (RTP), Nucleoside

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triphosphatase (NTPase) and a helicase. NS3 aids in viral replication and processing of polyproteins into structural and non-structural components (Bazan and Fletterick, 1989; Miller *et al.*, 2010). NS5 is the largest protein measuring approximately 103KDa and has three enzymes namely: N-terminal S-adenosyl methionine methyltransferase (MTPase), nuclear localisation sequence and RNA-dependent RNA polymerase. It role includes transportation of protein to nucleus, guanine 7- N- and ribose 2'-O-methylation and synthesis of new vRNA genome (Miller *et al.*, 2010).

The 5'UTR contains 95-135 nucleotides and a type I cap (Yu *et al.*, 2008). The cap structure is responsible for the cap dependent translation initiation of viral RNA by scanning 5'UTR for initiation codons. The 3' UTR contains 114-650 nucleotides and folds into a stem loop (SL) but lacks a Poly (A) tail. A sequence that is conserved is found upstream of the SL (Men *et al.*, 1996). This sequence contains a cyclization sequence (CS) which has a complementary sequence at the 5' end of the genome (Hahn *et al.*, 1987). Both untranslated regions are vital for replication and translation (Gamarnik, 2010; Padmanabhan and Strongin, 2010). Aside the cap structure at the 5' UTR, there is also a large stem loop (SL) which act as a promoter for RNA polymerase-methyl transferase NS5 (Yu *et al.*, 2008). The 5' and 3' UTR have complementary upstream AUG regions (UR) and cyclization sequence regions which hybridize during genome cyclization and RNA synthesis (Gamarnik, 2010).

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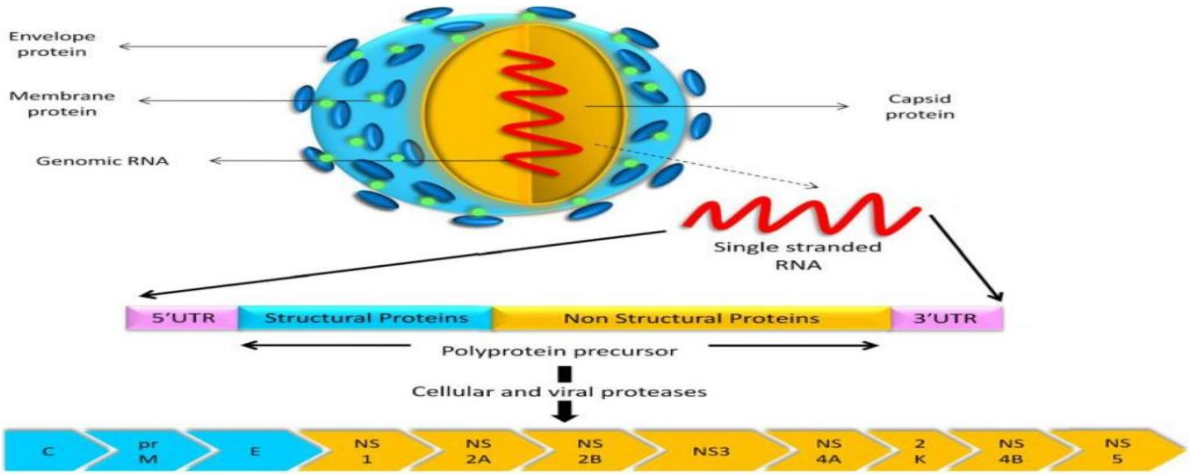

Figure 2.1: Schematic diagram showing the structure of a Zika virus

Source: (Guzman *et al.*, 2010)

2.1.2 Replication Cycle of Zika Virus

Replication of Zika virus takes place in the salivary gland and midgut of the *Aedes* spp. of mosquitoes (Li *et al.*, 2012; Wong *et al.*, 2013). After viral inoculation by *Aedes* spp. of mosquitoes through a blood meal, the virus enters the skin fibroblasts, keratinocytes, undeveloped dendritic cells (Hamel *et al.*, 2015), neurons and astroglial cells (Bell *et al.*, 1971) using adhesion factors such as AXL receptor tyrosine, DC-SIGN (Dendritic Cell-Specific Intercellular adhesion molecule -3- Grabbing Non-integrin) and varied members of the phosphatidylserine receptor family (Hamel *et al.*, 2015). Replication of Zika virus takes place near the inoculation site and later spreads to the blood and lymph nodes.

After entry, viral particle is internalized in the cell and the viral genome is released inside the cytoplasm. This is achieved when the viral envelope merges with the membranes of the cellular endosomes of the host cell due to the acidic pH inside the cellular endosomes

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(Stiasny *et al.*, 2011; Vazquez-Calvo *et al.*, 2012). Viral RNA acts as mRNA and negative and positive strands of viral RNA are synthesized. Newly formed positive strand-RNA is packed into progeny virions and bud off into the endoplasmic reticulum to form enclosed immature virions. Immature virions are transported via the Golgi complex and the prM is cleaved (Hayes 2009). The virus replicates and spreads further to infect other parts of the body such as myocardium, central nervous system, skeletal muscles and to the fetus (Chan *et al.*, 2016). The replication cycle of Zika virus is depicted in figure 2.2.

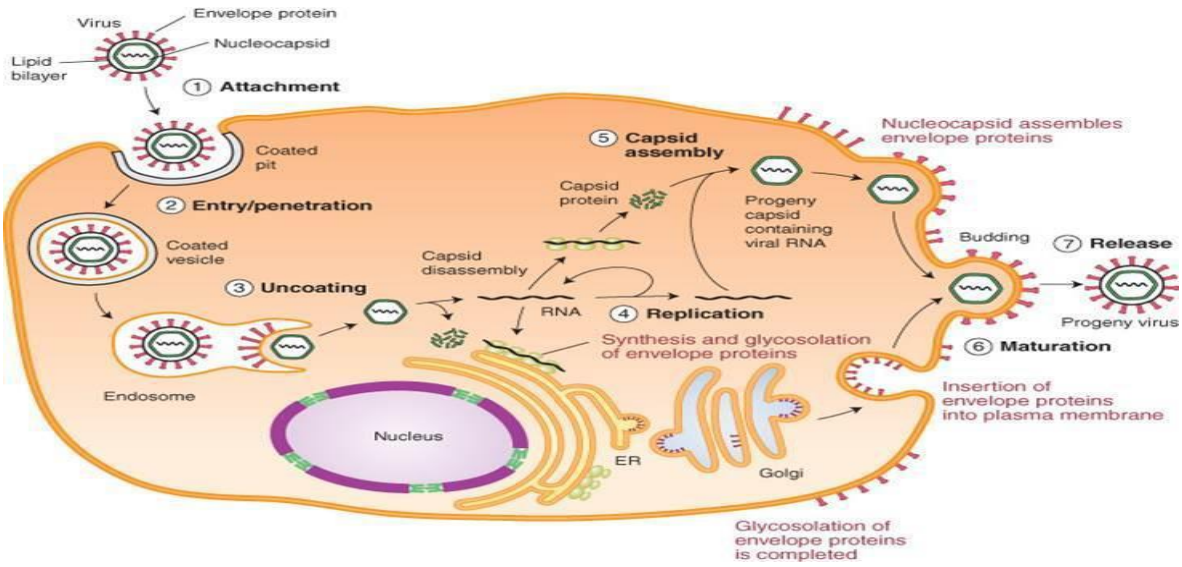

Figure 2.2: Replication cycle of Zika virus

Source: Chan *et al.*, (2016)

2.2 Epidemiology of Zika Virus Infection

Zika virus infection is among some of the fastest spreading arboviral infections with an estimated 30 percent growth in the number of reported cases since its inception in 1947 in Uganda (Dick, 1952). Most affected continents are Oceania and America with some parts

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of Africa and Asia showing high prevalence of the infection (Hill *et al.*, 2017). Roughly two billion individuals live in Zika virus infection endemic areas with 390 million infected individuals showing symptoms of Zika virus infection annually (World Health Organization, 2009). An estimated 99 million individuals develop symptoms for the different levels of disease severity (Bhatt *et al.*, 2013).

Transmission of Zika virus infection exists as forest and urban cycles. Forest transmission cycle involves Rhesus monkeys and forest species of *Aedes* mosquitoes whereas urban transmission cycle involves human–mosquito–human. Zika virus is maintained in the forest cycle with cyclic epizootics in monkeys. Humans are accidently infected by the forest cycle (Martin- Acebes and Saiz, 2012). In the transmission cycle of Zika virus, Rhesus monkeys are the reserviors with intermittent involvement of humans (Haddow and Dick, 1948; Haddow *et al.*, 1964). However, in areas without Rhesus monkeys, humans serve as the main reservior (Haddow *et al.*, 2012).

Zika virus is transmitted by mosquitoes of the *Aedes* (*Stegomyia*) genus. Zika virus is mainly transmitted by *Aedes aegypti*, *Aedes africanus*, *Aedes hensilli and Aedes albopictus* (Dick *et al.*, 1952). *Aedes aegypti* is the main vector involved in the epidemic that occurred in Asia and the French Polynesia (Olson *et al.*, 1981 and Oehler *et al.*, 2014). Although Zika virus has not been detected in *Aedes hensilli* and *Aedes polynesiensis*, they have been reported to be the main vectors in the Yap outbreak (Musso *et al.*, 2014). In Africa, the predominant vector is *Aedes albopticus* (Grard *et al.*, 2007). In Africa and Asia, other vectors involved are *Aedes africanus*, *Aedes luteocephalus*, *Aedes furcifer* and *Aedes Taylori*. *Aedes aegypti* and *Aedes albopictus* are responsible for the epidemic in the Americas (Ledermann *et al.*, 2014).

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Aedes aegypti and *Aedes albopictus* bite during the day and are commonly found in the tropical and subtropical regions. *Aedes aegypti* and *Aedes albopictus* have intrinsic ability to transmit the Asian genotype of Zika virus strain. The ability of *Aedes aegpti* to transmit Zika virus in a given location is because they live in close association with human, feed mostly on humans, bite multiple humans in a single blood meal and have an almost imperceptible bite (Dick *et al.,* 1952; Marchette *et al.,* 1969). *Aedes albopictus* is found in more temperate regions compared to *Aedes aegypti*, thus extending the possible range where epidemics can occur. *Aedes aegypti* is distributed throughout America, Asia, Oceania, Europe and Africa (Kraemer *et al.,* 2015) and can be shielded in local settings. Meanwhile *Aedes albopictus*, the most aggressive species of *Aedes* mosquitoes (Medlock *et al.,* 2012), have extended it territory to North, Central and South America, Africa, southeastern Asia, China, Japan, northern Australia, and southern Europe in the last 30–40 years (Paupy *et al.,* 2009).

This success is as a result of the ability of *Aedes albopictus* to adjust to different climates through the production of cold-resistant eggs. Preference of *Aedes albopictus* to containers in domestic settings has also increased it contact with humans (Medlock *et al.,* 2012). Zika virus has uncommonly been isolated from *Aedes unilineatus, Anopheles coustani* and *Mansonia uniformis* mosquitoes (Dick *et al.,* 1952).

Zika virus infection can be transferred from mother to fetus. Viral RNA and antigens have been isolated from amniotic fluids, placentas and brain tissues of mothers whose children were diagnosed with cerebral abnormalities and microcephaly (Besnard *et al.,* 2014; Musso *et al.,* 2015). Peripartum transmission of Zika virus infection involving mother and infant has also been reported (Besnard *et al.,* 2014; Musso *et al.,* 2015). Although transmission of

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Zika virus infection through sexual intercourse has been documented, risk factors and duration have not been determined.Viral particles and RNA often in high quantities have been identified in sperms up to 62 days after the onset of symptoms (Besnard *et al.,* 2014; Musso *et al.,* 2015).

Given the transmission routes of other arboviral infections, transmission of Zika virus through blood transfusion is likely to occur even though it has not been reported. An individual became infected with Zika virus after been biten by a monkey in Indonesia (Besnard *et al.,* 2014; Musso *et al.,* 2015). Two infections in laboratories have been reported. Zika viral RNA has been found in the breast milk of a symptomatic woman on the day of delivery (Besnard *et al.,* 2014; Musso *et al.,* 2015).

In a study carried out at the Colombia-Venezuela border, 29 (18.47%) of the 157 sera sampled from febrile patients were positive for Zika virus by real-time PCR. Prevalence of Zika virus co-existing with dengue and chikenguya was 6.37% and 5.10% respectively. Zika virus was also detected in a 27-year-old man who sought treatment at the Jambi city hospital 2 days after a sudden onset of fever in Indonesia (CDC, 2016). Zika virus was diagnosed as the cause of fever in a 52-year-old woman after a visit to El Salvador (Teale *et al.,* 2016).

Anti-Zika virus IgM 17 (8.4%) and IgG 120 (59.1%) were detected from 203 sera sampled in 2000 in Senegal. Fourteen out of the 17 anti-Zika virus IgM positives were confirmed by seroneutralization test (Fatim *et al.,* 2000).

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In 2014 in Puerto Rico, out of the 29,345 Zika virus infected individuals reported, the highest incidence occurred among persons aged between 20 and 29 years. Subsequently in 2015–2016, of the 28,341 Zika virus infected individuals documented, the median age was 32 years. Incidence increased in persons aged 20 –29 years and 10–19 years when pregnant women were included (Matthew *et al.,* 2016). Outbreak in the French Polynesia recorded 383 serologically confirmed Zika virus infected individuals of which prevalence was higher in 28 years old individuals (Paixão *et al.,* 2016).

According to Matthew *et al.,* (2016), reasons for the higher number of individuals infected with Zika virus aged 20 years and above are unknown. In Bahia state and El Salvador, age group 20–49 years were the most affected (Dos Santos *et al.,* 2016). During the 2007 epidemic in the Yap State, the attack rate among confirmed cases of Zika virus infection peaked among persons aged 30–39 years (Duffy *et al.,* 2009).

Zika virus infection is more prevalent in females than males (Paixão *et al.,* 2016). In Puerto Rico, among the 28,219 patients confirmed with Zika virus infection, frequency was greater among women compared to men for all age groups. Even though prevalence of Zika virus infection in females age group 10–19 years was similar the observation in males of the same age group, prevalence in females persistently escalated with age and peaked among women aged 3 to 39 years (Matthew *et al.,* 2016).

Similarly, in Bahia state and El Salvador, total number of individuals infected with Zika virus was 75% higher in females than in males (Dos Santos *et al.,* 2016). Outbreak of Zika virus infection in the Yap State of Micronesia recorded 61% of females' infected (Duffy *et al.,* 2009).

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Rate of infections has increased at different epochs. In Southern Cone and Andean, transmission of Zika virus infection was intensified in January, peaked in February and decreased (Ikejezie *et al.,* 2016). Again, in the Central America, rates escalated in January and June (Ikejezie *et al.,* 2016). Non-Latin Caribbean sub-regions recorded high rates of Zika virus infection in the months of February and June respectively (Ikejezie *et al.,* 2016). In the Latin Caribbean sub-region, where prevalence of Zika virus infection is at its peak, rates increased in 2016, and continued at high intensity throughout the month of July (Ikejezie *et al.,* 2016).

These differences are associated with temperature, rainfall and humidity (Brisbois *et al.,* 2010). In Indonesia a maximum rainfall of 2463 mm and minimum of 16.8 mm with an average rainfall of 352.2mm are appropriate for Zika virus transmission (Gharbi *et al.,* 2011). A link between the incidence of Zika virus infection and rainfall has also been established (Ariati *et al.,* 2012).

2.2.1 Zika Virus Infection in Africa

Despite the presence of Zika virus infection in Africa since 1947 (Dick, 1952), limited data exist on the prevalence especially in febrile patients. This has been attributed to the sporadic nature of the infection with overlapping signs and symptoms resembling other infectious diseases, misdiagnosis and limited availability of tools for testing (Foy *et al.,* 2011). Zika virus infection was first documented in 1947 in a rhesus macaque monkey and 1948 in an *Aedes africanus* mosquito in Uganda (Dick, 1952). Subsequently in 1952, Zika virus infection was found in humans in Tanzania and Uganda (Haddow *et al.,* 2012).

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In 1954, a 10 years old female who was reported to a hospital in Nigeria was diagnosed with Zika virus infection after several laboratory tests (Macnamara, 1954). Other two cases reported in 1954 in Nigeria confirmed the possible circulation of Zika virus (Macnamara, 1954). In 1964 and 1975, Nigeria became endemic especially in Ibadan (Moore *et al.*, 1975). Although, Zika virus infection was found in all age groups, children below 4 years were the most affected (Moore *et al.*, 1975). Later, the infection was found among people in Kenya (Geser *et al.*, 1970), Gabon (Jan *et al.*, 1978; Grard *et al.*, 2014), Central African Republic (Saluzzo *et al.*, 1981), Sierra Leone (Robin and Mouchet, 1975), Senegal (Monlun *et al.*, 1993; Diallo *et al.*, 2014; Althouse *et al.*, 2015), Uganda (McCrae and Kirya, 1982) and Ivory Coast (Akoua-Koffi *et al.*, 2001), with a prevalence level ranging between 1.3–52%.

Higher number of individuals with Zika virus infection was recorded in 1969 as compared to 1965 and 1967. Prevalence of Zika virus infection in 1970 in Kenya was attributed to the higher housing density (Geser *et al.*, 1970). Growing age, farming and grass roof were the associated risk factors for the occurrence of Zika virus infection in Zambia. However, indoor residual spraying and having an iron roofing sheet over the house reduced the risk for Zika virus infection (Babaniyi *et al.*, 2015).

From 2008 to 2013, three people were diagnosed with Zika virus infection in Senegal (Heang *et al.*, 2012; Heang *et al.*, 2011). In 2016, Zika virus infection was transmitted locally in Guinea Bissau and Angola (World Health Organization, 2016 and 2017). The table below gives a brief description of the prevalence of Zika virus infection in Africa since 2000 (Waddell and Greig, 2016).

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Table 2.1: Prevalence of Zika virus infection in Africa since 2000

Country	Date	Population sampled	IgM and IgG Prevalence
Gabon	2007-2010	All ages-hospital submissions	0.1%
Senegal	2009-2013	Adult-clinic submission	0.1%
Cameroon	2010	All ages-hospital submissions	38%
Zambia	2010	General population	6%
Cape Verde	2010-2016	General population	Outbreak with 19,083 cases
Ethiopia	2013	Hospital submissions	10%
Kenya	2013	General population	0.0%

Source: Waddell and Greig, (2016)

2.2.2 Zika Virus Infection in Asia

First reported cases of Zika virus infection in Asia was in India (Smithburn, 1954). Afterwards, clusters of Zika virus infection were found between 1977 and 1980 in Malaysia, Indonesia and Pakistan toward the end of the raining season (Olson *et al.*, 1981; (Darwish *et al.*, 1983). Zika virus infection was subsequently detected in Sabah, Malaysia in 144 orangutans (Kilbourn *et al.*, 2003).

In 2010, few individuals were sporadically infected with Zika virus infection in Cambodia (Heang *et al.*, 2012). Because of the outbreak in the Americas, alertness and observation for

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Zika virus infection in Asia was improved and this resulted in better recognition and reporting. Rigorous analysis of sera from Cambodia, Bangladesh and Lao People's Democratic Republic identified patients who were initially diagnosed as negative for Zika virus infection (WHO, 2017 and Duong *et al.*, 2017).

Implementation of improved surveillance for Zika virus infection in Thailand in 2016 increased the total number of Zika virus infections reported (WHO, 2016). A sero-epidemiological study found proof of Zika virus infection in some parts of Thailand (Wikan *et al.*, 2016). Zika virus infection was also reported in tourists returning from Thailand (Ellison *et al.*, 2016). Evidence of possible circulation of Zika virus was provided in Singapore in 2016 and 2017 (Leo and Chow, 2016). Zika virus infection was also found in South Korea in 2016 (Leo and Chow, 2016).

2.2.3 Zika Virus Infection in Oceania

After the emergence of Zika virus infection in 1947, Zika virus infection showed a geographical restriction (Duffy *et al.*, 2009). Zika virus infection was confined to Africa and Asia until 2007 when evidence of exposure was found on the island of Yap in the Federated States of Micronesia with about 5000 infected individuals. The occurrence of Zika virus infection in the Yap State of Micronesia was initially misdiagnosed as Dengue, Chikungunya or Ross River disease. However, further analyses revealed Zika virus RNA in the sera of these patients. About 49 individuals were confirmed with Zika virus infection with no hospitalizations or deaths (Duffy *et al.*, 2009). The infection was predominant in children aged 3 years (Duffy *et al.*, 2009).

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Epidemic of Zika virus infection in the French Polynesia was in 2013. Cases were reported from Marquesas and Tuamotu Islands in Mo'orea, Tahiti, Tahaa, Raiatea, Bora Bora, Arutua, Nuku and Hiva. Initially, it was assumed as Dengue with approximately 19,000 cases. By 2014, cases had increased to 29,000 with 8,503 suspected as Zika virus infection. Outbreak declined in March 2014, abating in October 2014 with approximately 30,000 Zika virus infected individuals (Derraik and Staney, 2015). Outbreak lasted for 21 weeks with an estimated 3,500 individuals complaining of fever. Actual number of infections in the French Polynesia was not known because patients did not seek medical care (Aubry *et al.*, 2015).

Zika virus infection also occurred in New Caledonia with 64 cases reported from Dumbea, Noumea and Ouvea of which thirty were introduced from the French Polynesia. By 2014, 140 individuals were infected with Zika virus with thirty two imported. Outbreak increased in April and by September, number of individuals with Zika virus infection had increased to 1,400 with thirty five imported. Zika virus infection circulated in the country until 2015 with 137 confirmed cases. Outbreak lasted for 29 weeks (European Centre for Disease Prevention and Control, 2015).

In Cook Island, outbreak occurred in February 2014 with eighteen confirmed and two imported cases (European Centre for Disease Prevention and Control 2014). Outbreak ended in May with fifty cases confirmed as Zika virus infection and 932 suspected as Zika virus infection (WHO, 2014). Forty individuals were suspected to have Zika virus infection in 2014 in the Easter Island. Fifty individuals were infected with Zika virus in Chile (Tognarelli *et al.*, 2016). These individuals were suspected to have been infected in the French Polynesia during the annual Tapati festival (Musso, 2015). Zika virus infection was

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also reported in Vanuatu weeks after the tropical cyclone in 2015 (European Centre for Disease Prevention and Control, 2015).

2.2.4 Zika Virus Infection in America

About 175,063 individuals have been infected with Zika virus in the Americas since the epidemic in 2015 (Brazil Ministry of Health, 2016). Epidemic of Zika virus infection in America first occurred in Brazil and Colombia. Brazil is the most affected country with approximately 500,000 to 1,500,000 individuals (Bogoch *et al.,* 2016). Case counts for Zika virus infection was not available in Brazil until 2016 when Zika virus infection became a national notifiable condition (Brazil Ministry of Health, 2016). Most of the individuals infected with Zika virus were native of Natal. Afterwards, Zika virus infection was found in individuals of Brazil, Guatemala, Colombia, Mexico, El Salvador, Paraguay, Bonaire, Samoa, Aruba, Sint Maarten, Trinidad and Tobago, Argentina, Venezuela and USA (Corsica, 2015).

2.3 Conditions for Spread of Zika Virus Infection

2.3.1 Climate Change and Variation

The geographical distribution of *Aedes* spp. of mosquitoes has been exaggerated by climate variation and change. Climate change and variation have enlarged temperatures to a level that favour transmission and lengthen the transmission season in the tropical and subtropical regions. Temperature affects incubation and fecundity rates, survival, development and biting of *Aedes aegypti* and *Aedes albopictus* (Mordecai *et al.,* 2016).

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The occurrence of Zika virus infection in the Americas has been ascribed to temperatures appropriate for *Aedes aegypti* (Hales *et al.*, 2002). Warmer winter temperatures have enlarged the number of *Aedes aegypti* breeding sites in Brazil (Stewart *et al.*, 2013).

Climate variation and change have become a great worry in the temperate regions where temperatures were not always suitable for *Aedes aegypti*. Warmer temperatures recorded in the winter have increased the survival rate of the *Aedes aegypti*'s egg that was formally restricted to 10°C, thereby expanding the geographical range of *Aedes aegypti's* (Foote *et al.*, 1961; WHO, 2009).

Warmer temperatures have also expanded the extent of transmission in the temperate regions especially in areas where *Aedes aegypti* or *Aedes albopictus* were locally established (Mordecai *et al.*, 2016).

2.3.2 Social Change and Urbanization

Aedes aegypti and Aedes albopictus are spreading to new countries due to human behaviors such as economic enterprises and expansion, trade and economic globalization, urbanization, population growth and poor sanitation (Simmons *et al.*, 2012). Social changes such as natural disasters increase human contact with Zika virus-infected *Aedes* spp. of mosquitoes. In Ecuador, increased local transmission was linked with a tremor that hit the Manabi province in 2016, contaminating drinking water, destroying infrastructure and forcing people to live outdoors (Mis, 2016).

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Disasters also interfere with educational programs, health delivery and vector control programs (Watson *et al.*, 2007). Apart from this, socioeconomic and political changes also expose people to poor infrastructure, water access and sanitation. Fluctuating vector control efforts, human movement and public sanitation policy further exacerbate present differences in these services (Watson *et al.*, 2007).

2.4. Impact of Zika Virus Infection

2.4.1 Public Health Impact

Because the infection has been found outside Africa and Asia, the virus is considered as an evolving pathogen. Zika virus infection up to date has been mild and benign until 2007 when severe forms of the infection were observed (Petersen & Hayes, 2004).

It discovery in Micronesia and the neurological abnormalities such as Guillian Barre Syndrome and microcephaly that were associated with the virus proves that the virus has evolved and can spread across large geographical areas. Diagnosis is difficult due to the cross-reactive antibodies produced by these flaviviruses and the overlapping symptoms and signs that resemble dengue and other flaviviral infections.

Given its overlapping signs and symptoms, prevention and control strategies include the use of insect repellent and implementation of interventions that will reduce the number of potential mosquito vectors. The rapid spread of Zika virus infection and its impact on health requires the cooperation of medical professionals, public health officers and first-class reference laboratories (Duffy *et al.*, 2009).

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2.4.2 Socio-economic Impact

Socioeconomic impact has been anticipated to be long-term, most likely to affect the poorest and helpless countries, and also widen inequalities in the world. The cost of the recent spread to Latin America and the Caribbean has been projected at 7-18 billion US dollars, impact that is more than what was recorded in South America. Apart from this, more than 80 percent of revenues annually earned from tourism were reduced, with the possibility of reaching a total of US$9 billion. Haiti and Belize are estimated to loss about 1.13% and 1.19% of GDP annually (UNDP and IFRC, 2017).

Aside revenue loss, the magnitude of stress that is likely to be placed on health care facilities can retard social development. Severest impact on development will be felt by the poorest economies in the world. In these regions, income that is estimated to be lost is projected at a half a billion dollars. Although several intensive efforts by most affected nations have been put in place to control the spread of Zika virus infection, these efforts have been faced with several challenges (UNDP and IFRC, 2017).

These include allocation of resources and coordination, prevention, surveillance and diagnosis. Social inequalities and inadequate health service coverage is a challenge for national response teams to reach the most helpless groups (UNDP and IFRC, 2017).

Furthermore, huge investment is required in durable control measures such as vaccine development, production of mosquito repellants, waste management, epidermiologic and entomologic researches to define the range of Zika virus vectors and identify new areas where autochthonous transmission could take place to enable early intervention. Cost of diagnosis is expected to high particularly in countries where resources for diagnosis are

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lacking and potential intervention measures (e.g., contraception or termination of pregnancy) discouraged or illegal (Fauci *et al.*, 2016).

2.5 Clinical Manifestation of Zika Virus Infection

Gestation period of Zika virus infection is three to twelve days and indications are usually mild and asymptomatic (Majumder *et al.*, 2016). Symptoms are relieved within two to seven days but virus can still be detected in the sera of the infected person after one week of cure. Severe clinical forms of the infection and mortality rates are very low. Persons develop immunity after exposure (Ahmad *et al.*, 2016). Key complications include microcephaly and Guillain–Barré syndrome

2.5.1 Adults

About 20–25 % of individuals infected with Zika virus develop Zika virus disease (Duffy *et al.*, 2009). Symptoms include mild fever with a tempereature greater than 37 °C, puritic rashes, headache, non-purulent conjunctivitis, myalgia, arthralgia (Brasil *et al.*, 2016), hematospermia (Musso *et al.*, 2015), asthenia, transient hearing loss (Tappe *et al.*, 2015), retro-orbital pain and subcutaneous bleeding (Karimi *et al.*, 2016).

Other symptoms include mucous membrane ulceration, lymphopenia, diarrhea, neutropenia, thrombocytopenia, nausea, monocytosis, increased erythrocyte sedimentation rate, thrombocytopenia (Zammarchi *et al.*, 2015), raised serum levels of gamma-glutamyl transpeptidase, lactate dehydrogenase, aspartate aminotransferase, abdominal pain, pruritus,

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ferritin, fibrinogen, transient and mild leucopenia and C-reactive protein during the course of the viremic phase (Dupont *et al.*, 2014).

Outburst of Zika virus infection in the Americas was characterized by an unusual increase in Guillain–Barré syndrome. However, the casual link was unknown (Mlakar *et al.*, 2016). During the outbreak of Zika virus infection in the French Polynesia, Zika virus infection was associated with Guillain–Barré syndrome. Anti-Zika virus IgM was positive in 93 % of Guillain–Barré syndrome cases (Cao-Lormeau *et al.*, 2016).

2.5.2 Children

Children are infected with Zika virus through the intrauterine, intrapartum and postnatal routes (Hennessey, 2016). Intrauterine consequences of Zika virus infection and its determinants are not fully known. Although the virus has been isolated from several cases of microcephaly, actual number of cases caused by Zika virus is unknown (Mlakar *et al.*, 2016). Symptoms include low birth weight, polyhydramnios, redundant scalp skin, arthrogryposis and anasarca. Neurological deformities include cerebral lesions, brainstem dysfunction and polymalformative syndromes (WHO, 2015).

Intracranial calcifications are mostly seen over the white matter of the lentostriatal vessels, frontal lobes, cerebellum, vermis and thalami, caudate, around the ventricles and asymmetrical cerebral hemispheres (Schuler-Faccini, 2015).

Symptoms in the later stages of pregnancy include cerebral atrophy, enlarged cisterna magna, dysgenesis of corpus callosum, severe unilateral ventriculomegaly and thinning of

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the parenchyma on the dilated side, pons and brainstem, sensorineural deafness, mental retardation and ophthalmological lesions (Schuler-Faccini, 2015). During the Brazil epidemic, there were 35 microcephaly cases (Schuler-Faccini, 2015). Heads have circumferences ≥2 standard deviations (Schuler-Faccini, 2015).

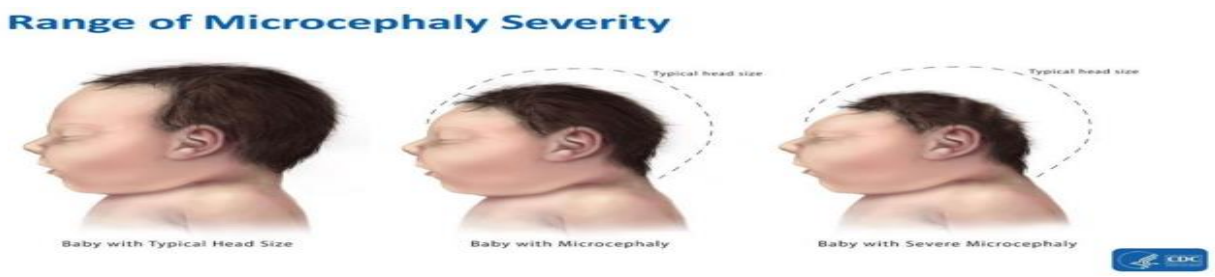

Figure 2.3: Range of microcephaly severity

Source: Lyle *et al.*, (2016)

2.6 Diagnosis of Zika Virus Infection

2.6.1 Case Definition

Zika virus infection is a self-limiting infection with patients recovering after a non-severe clinical course (Tripathi *et al.*, 2011). However, if Zika virus infection is not properly managed it can lead to severe forms of the infection such as Microcephaly and Guillian Barre syndrome. The WHO Zika virus infection case definition was therefore introduced for the timely identification and management of Zika virus infection by clinicians. This saves resources and leads to a reduction in Zika complications (Horstick *et al.*, 2014). Definitions according to the WHO, (2016) include;

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Suspected case: a person who has fever and/or rashes with either non-purulent conjunctivitis or arthralgia

Probable case: a person who has anti-Zika virus IgM with no proof of any other aboviral infection, and has not had any interaction with an infected person or resided in or travelled to an area with active transmission of Zika virus infection two weeks before onset of symptoms.

Confirmed case: is a person who has antigen or Zika virus RNA and a plaque reduction neutralization test titre value of ≥20 for Zika virus and titre ratio ≥ 4 compared to other flaviviruses

2.6.2 Laboratory Investigation

Investigation of Zika virus infection is often based on defined criteria for case definition and travel history (Rigau-Perez *et al.*, 1994). Clinical evaluation of Zika virus infection is undertaken concurrently with CHIKV and DENV for patients diagnosed with fever, myalgia, rash or arthralgia. Laboratory investigation of Zika virus infection can be made by one of the following methods;

- Cell culture to isolate virus (Vordam and Kuno, 1997)
- Real time PCR to identify viral nucleic acid
- Serology to detection of virus specific antibody

Viral detection (virus isolation, genome detection and antigen detection) is done at the acute phase (0-5) of the infection in plasma, serum, whole blood and tissues following the

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onset of symptoms. However, detection of the virus and its components from samples are not routinely employed in laboratories due to the few commercial kits available. As a result, commonly diagnostic tools routinely employed for the diagnosis of Zika virus infection are serological tests (Peeling *et al.*, 2010).

2.6.2.1 Virus Isolation

Common methods used in the isolation of Zika virus is the inoculation of serum into *Aedes albopictus* cell lines (C6-36) and *Aedes pseudoscutellaris* (AP61) (Guzman and Kouri, 1996; Race *et al.*, 1979), Vero cells and LLC-MK2 and inoculation into intra-cerebra of suckling mice (Peeling *et al.*, 2010). Isolation of Zika virus from larval or adults of *Toxorchunchitis spp* mosquitoes (*T. ambionesis*, *T. splendens*) (Gubler and Rosen, 1976; Rosen and Shroyer, 1985) has a higher viral isolation rate of 80% as compared to other methods (Vordam and Kuno, 1997).

Immunofluorescent assay is performed to identify the virus, either seven to ten days after inoculation in cell lines or fourteen days after inoculation in mosquitoes (Guzman and Kouri, 1996; Henchal *et al.*, 1983; Vordam and Kuno, 1997). Culturing of Zika virus has a lot of limitations. It requires specimen from patients within two to seven days of infection (with sufficient viral loads) and isolating the virus immediately as the period for successful isolation of the virus is short. Culturing of Zika virus is time consuming, labour intensive and requires training (World Health Organization, 1997).

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2.6.2.2 Viral RNA Detection

Viral RNA can be isolated from tissues and sera using molecular amplification methods. Molecular amplification (e.g., RT-PCR) is the preferred method for diagnosing Zika virus infection due to its high sensitivity and specificity. Currently, there are seven real-time RT-PCR assays for Zika virus RNA detection (Waggoner and Pinsky, 2016). Protocols developed for the isolation of Zika viral RNA employ the use of primers that target specific regions in the genome such as membrane (M), envelop (E) and the nonstructural proteins (NS) 1, NS2, NS5 and NS3 (Lanciotti *et al.*, 1992; Johnson *et al.*, 2005; Wu *et al.*, 2001).

2.6.2.3 Serological Diagnosis

Serological methods used for diagnosing Zika virus infection include ELISA, indirect immunofluorescence assays (IIFA) and virus neutralization tests (VNT) (Guzman and Kouri, 1996). Furthermore, these assays can only detect antibodies five days after the onset of disease, making them unsuitable for rapid diagnosis (Guzman and Kouri, 1996).

2.6.2.3.1 Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA is the best tool for detecting anti- Zika virus IgG and IgM antibodies during the acute phase of Zika virus infection. They include the IgM based assays, IgG based assays or rapid immunochromatographic test (Guzman and Kouri, 1996; Vordam and Kuno, 1997; Innis *et al.*, 1989).

Anti-Zika virus IgM is detectable four to five days after the onset of symptoms and can remain detectable 2-3 months. Positive result for anti-Zika virus IgM can be suggestive of a current infection. However, in an endemic area, demonstration of a fourfold increase in antibody titres of paired samples is required to diagnose a current infection (World Health Organization, 2009). Anti-Zika virus IgG ELISA kits are employed to establish a previous

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exposure to Zika virus (Peeling *et al.*, 2010). During a primary infection, anti-Zika virus IgG levels are often low but increases over time (Halstead and Papaevangelou, 1980).

Anti-Zika virus ELISAs has microplates treated with anti-human IgM or IgG antibodies to bind the equivalent antibodies in the sample. After washing the well, horseradish peroxidase (HRP) labeled Zika virus antigen is added. This antigen-conjugate binds to the captured anti-Zika virus IgG or IgM antibodies. In a second washing step unbound conjugate is removed and Tetramethylbenzidine (TMB) substrate is added which gives a blue reaction product. The resultant colour change after adding stop reagent is quantified by a spectrophotometric reading of optical density (OD) which is directly proportional to the amount of antigen-specific IgM or IgG present in the sample. The samples OD reading is compared to a reference cut-off value to determine results (Peeling *et al.*, 2010).

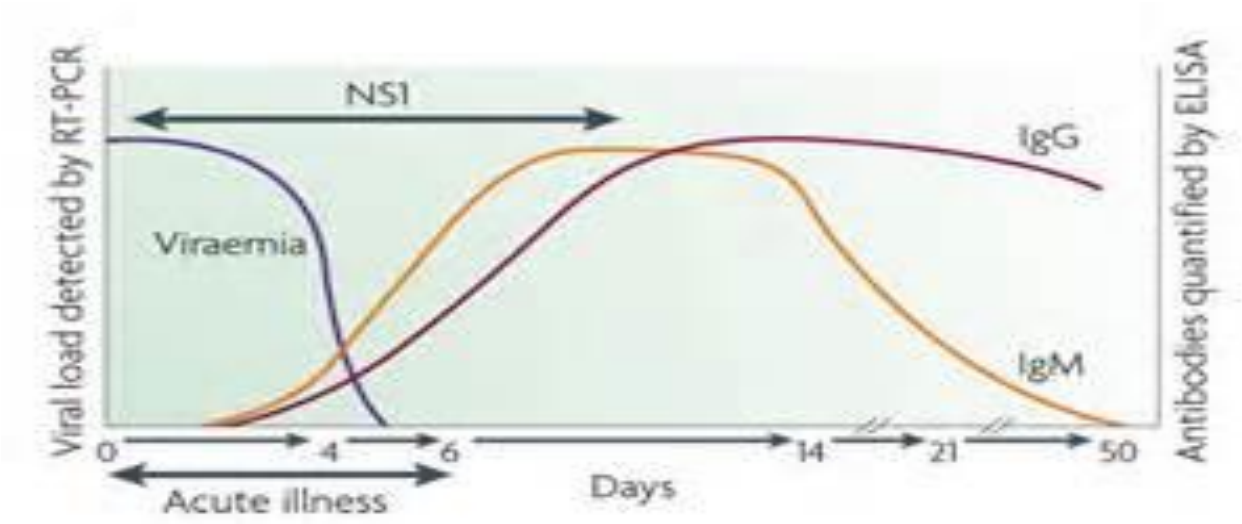

Figure 2.4: Major diagnostic markers for Zika virus infection

Source: Peeling *et al.*, (2010).

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2.6.2.3.2 Plaque Reduction Neutralization Test

It is recommended that presumptive positives, equivocal or unconvincing IgM test results be confirmed by this method. Sera or solution of antibody is diluted and mixed with a constant volume of Zika viral suspension. This is incubated and poured over a confluent monolayer of host cells. The surface of the cell layer is covered in a layer of agar or carboxymethyl cellulose to prevent the virus from spreading indiscriminately. The concentration of plaque forming units is estimated by the number of plaques (regions of infected cells) formed after a few days (Schmidt *et al.*, 1976).

Vital dye such as neutral red is then added for visualization of the plaques and the percentage neutralization estimated by dividing the quantity of plaques formed in individual plate by the original number of virions. The plaque forming units are measured by microscopic observation, fluorescent antibodies or specific dyes that react with infected cells. The concentration of serum to reduce the number of plaques by 70% compared to the serum free Zika virus gives the measure of how much antibody is present or how effective it is (Schmidt *et al.*, 1976).

Currently it is considered to be the "gold standard" for detecting and measuring antibodies that can neutralize Zika virus (Thomas *et al.*, 2009-11; Ratnam *et al.*, 1995). It has a higher sensitivity and specificity than Enzyme immunoassay. However, the test is relatively cumbersome and time intensive (few days) relative to ELISA kits that give quick results (usually several minutes to a few hours) (Schmidt *et al.*, 1976).

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2.6.2.3.3 Indirect Immunofluorescence

In indirect immunofluorescence assay, two antibodies are used in the diagnosis of Zika virus infection. A primary antibody which has not been labeled binds specifically to the antigen. A secondary antibody which has been labeled with a fluorophore recognizes the primary antibody and binds to it. This provides signal amplification by increasing the number of fluorophore molecules per antigen (Fritschy and Hartig, 2001).

2.7 Treatment and Prevention of Zika Virus Infection

2.7.1 Treatment and Therapeutic Approaches

Treatment of Zika virus infection is directed to symptoms relief with fluids, rest, anti-pyretics and analgesics (Engle and Diamond, 2003).

Acetaminophen is used to relief patients of headache, myalgia and fever. Pruritus is managed with antihistamines whiles fluid loss is fixed with adequate rehydration (Chen and Hamer, 2016). For effective treatment of latent neurological complications particularly Guillian Bare Syndrome, it is advised that diagnosis be done quickly. Treatment involves the use of immunoglobulins and plasmapheresis (Chen *et al.,* 2016). Infected children and family are supposed to be counseled by pharmacists, medical geneticists, neurologists and other health specialists (Staples *et al.,* 2016).

Currently, thirty-eight vaccines have been reported to the World Health Organisanition. Thirty two are in the pre-clinical stage, five in phase one trial and one in phase two trail. These vaccines include DNA, mRNA, inactivated and live attenuated vaccines, proteins

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and peptides (Lagunas-Raangel *et al.*, 2017). The table below (Table 2.2) shows some of the vaccines that have been developed and their trial stage.

Table 2.2: Current Zika vaccine candidates at various stages of preclinical/clinical trials

Vaccine Approach	Developer	Status
DNA-based vaccine	NIAID	Phase 2
Purified inactivated Zika vaccine	WRAIR	Phase 1
A live-attenuated vaccine	NIAID	Phase 1
mRNA vaccines	NIAID and GSK	Phase 1
AGS-v,	SEEK	Phase 1

Source: NIAID, (2017)

2.7.2 Prevention of Zika Virus Infection

Control and prevention of Zika virus infection is mostly focused on preventing the bites of *Aedes* spp. of mosquitoes. Methods include putting on long-sleeved shirts and pants, using insect repellent, minimization of outdoor activities, using screened windows and doors, elimination of standing water and enactment of mosquito control programs (Martin-Acebes and Saiz, 2012).

The European Center for Disease Prevention and Control and the Center for Disease Control and Prevention recommend that, pregnant women and those who want to get

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pregnant should not to travel to areas with active Zika virus transmission. Again, areas where Zika virus infection has not reported, but has potential mosquito vectors, it is recommended that a rapid response system be adapted by the public health institutions to prevent local transmission when a case is detected. The World Health Organisation on the other hand calls for collaboration between the health authorities and the transport sectors in various countries in order to disinfect any air craft travelling from affected areas (Musso *et al.,* 2015).

The Center for Disease Control and Prevention also endorses the use of condom when having sexual intercourse while traveling or if partner has just come from an area with active viral transmission. Furthermore, pregnant women are advised to abstain from sexual intercourse. Likewise, since Zika virus infection can be spread through blood transfusion, countries with or without active transmission of Zika virus infection are advised to take measures to prevent transmission through blood transfusion (Musso *et al.,* 2014).

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CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design and Ethical Consideration

3.1.1 Study Design

The study was a cross sectional study involving archived serum samples from febrile patients at the Greater Accra regional hospital between December 2016 and November 2017. These clinical specimens were obtained as part of an ongoing project with the aim of using serological and molecular tools to detect Dengue and Chikungunya in febrile patients within selected health facilities in Ghana. A structured case investigation form was used to collect information about demographic features and clinical symptoms of the febrile patients (Case Investigation Form, Appendix 1).

3.1.2 Ethical Consideration

This study used archived sera from an on-going project at Noguchi Memorial Institute for Medical Research, thus ethical clearance obtained for this ongoing project from the Noguchi Memorial Institute for Medical Research Institutional Review Board covered this work. Besides this, ethical clearance was also solicited from the College of Health Sciences Ethical and Protocol Review Committee, University of Ghana.

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3.2 Study Site and Sample Size Determination

3.2.1 Study Site

Greater Accra regional hospital serves as a secondary referral center as well as the main hospital for the Greater Accra Region. Catchment area of Greater Accra regional hospital is the whole Greater Accra region with a population of about 4,283,322 inhabitants. The hospital was selected for this study based on the high number of febrile cases recorded at the hospital and the presence of well-established clinical and laboratory facilities. The hospital has a 600-bed capacity and provides a wide range of services including out-patients, in-patient, specialist services and administration and support services.

3.2.1 Sample Size Determination

Sample size was determined using hospital admissions prevalence rate obtained from a study conducted by Tsegaye (2014) in Ethiopia in 2013. This is the only recent seroprevalence study conducted in Africa I found during my literature review, as a result it selection.

With 95% confidence level and a corresponding Z- value of 1.96;

Error margin of 0.05,

Prevalence (P) of Zika virus infection of 10% (0.10)

Sample size required was determined using the formula;

$$N=\frac{Z^2P(1-P)}{d^2}$$

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Sample size = $(1.96^2 x\ 0.10x\ 0.88)/0.05^2$ =135 samples

However, one hundred sixty (160) serum samples were used for this study because of the design of anti-Zika virus ELISA test kits used.

3.3 Eligibility Criteria

3.3.1 Inclusion Criteria

The study recruited all persons in all ages including pregnant women and children. For a consented participant to meet the inclusion criteria he/she should have fever (body temperature ≥ 38°C) and at least two of the following signs or symptoms: conjunctivitis, diarrhea, nausea, vomiting, muscle pain, joint pain, jaundice, loss of appetite, chest pain, rapid respiration and recent loss of hearing.

3.3.2 Exclusion Criteria

1. Patients positive for Malaria by blood film test

3.4 Sampling Procedure and Documentation

3.4.1 Sampling Procedure

Frozen sera were used for the study. Sampling procedure used for the collection of the sera is as follows;

Human whole blood samples were taken by nurses that have been trained on the case definition at the Greater Accra regional hospital.

From an eligible patient who had consented to be part of the study, a good-sized vein was identified, usually in the antecubital fossae or on the dorsum (back) of the hand. A

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tourniquet was then applied proximal to the site of venipuncture to ensure engorgement of vein with blood. The site of the venipuncture was cleaned with an alcohol swab and approximately 5 milliliters of blood is collected into a BD Vacutainer with SST II Advance Semi-separator gel (BD, Belliver Industrial Estate, Plymouth, PL6 7BP, United Kingdom), mixed by turning it 5 times upside-down. Once enough blood was withdrawn, the tourniquet was removed, with the needle still in place, a cotton swab was taken and placed over site of needle insertion and the needle was gently removed. Direct pressure was applied with the cotton swab over the puncture site to stop any bleeding (about 2 minutes), after which the swab was removed to ensure bleeding has stopped. If not, the swab was affixed with gauze tape.

3.4.2 Documentation

Participant who consented and their clinical samples taken also provided demographic information which were captured on a structured case investigation form (Appendix 1) as well as their clinical information. All the serum collecting tubes were labeled with unique study number that also corresponded with their identification number on the case investigation form.

3.5 Sample Processing and strategy

3.5.1 Sample Processing

Samples were processed into sera and cryogenized as follows;

The blood samples (approximately 5ml) collected were processed into serum at the virology department, Noguchi Memorial Institute for Medical Research. The processing involved the blood in the serum separator tube been centrifuged at 1000 × g for 10 minutes

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to separate the serum which usually will be about 3.5mls in volume. The serum was carefully removed and transferred aseptically using a fine-bore pipette into two separate sterile labeled 1.8ml-sized cryogenic vials. Aliquots of the serum were used in the laboratory investigation and the rest stored permanently under ultra-low (-80°C) freezing conditions at NMIMR. It was from these frozen sera, samples were taken to run for anti-Zika virus IgG and IgM.

3.5.2 Sampling Strategy

Every respondent who met the eligibility criteria and consented to the study was sampled. Recently collected será (between December 2016 and November 2017) were purposively collected from the banked será.

3.6 Laboratory Testing of Zika Virus Infection

All reagents and sera were allowed to thaw and mixed well before used according to Abcam anti-Zika virus IgM and IgG capture ELISA kits (Abcam, Cambridge, United Kingdom) protocols (Figure 3.1).

Abcam anti-Zika IgM and IgG capture ELISA kits contain Zika virus IgG and IgM microplate, IgG and IgM sample diluent, stop solution, 20X wash buffer solution, horseradish peroxidase (HRP) Zika virus conjugate, 3, 3’, 5, 5’-tetramethylbenzidine (TMB) substrate solution, Zika virus IgG and IgM positive control, Zika virus IgG and IgM cut–off control, Zika virus IgG and IgM negative control and cover foil.

Working washing solution was prepared by diluting 1 part (eg. 10ml) of the Washing solution with 19 parts (190ml) of fresh distilled water. Subjects’ sera were diluted with the

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dilution buffer specially prepared for testing for IgM and IgG antibodies to Zika as provided by the manufacturer. One part (10 µl) of serum was diluted with 100 parts (1ml) of the dilution buffer.

Microtitre strip wells were arranged according to the number of samples. One hundred microlitres of the negative control were aliquoted into wells C1 and D1 while well A1 and B1 were left blank according to the Abcam anti-Zika virus IgM and IgG capture ELISA kits protocols. One hundred microlitres of the cut-off and positive control were also aliquoted into wells E1 and F1 and G1 and H1. One hundred microlitres of diluted patients' serum were then aliquoted into each of the remaining microplate wells (80 of them). The wells were covered with a foil and incubated at 37ºC for an hour. After incubation, the foil was removed, content of wells aspirated and washed with 300µl of washing buffer. The wells were blotted on an adsorbent paper until completely dried. One hundred microlitres of HRP Zika virus conjugate (peroxidase labeled Zika virus antigen) were added to each well except for the substrate blank wells and incubated for 30 minutes at 37ºC. The washing procedure was repeated after which 100µl of substrate solution (3,3´,5,5´-tetramethylbenzidine (TMB) hydrogen peroxide) was added to each well and incubated in the dark at room temperature 20º C - 25º C for 15 minutes. One hundred microlitres of the stop solution was added to each well. The ELISA microtitre plate reader (Human Diagnostic Worldwide, Germany) (Figure 3.2) was set to zero using the substrate blank in wells A1 and B1 as shown in Table 3.1. The absorbance was measured at 450nm within 30 minutes after terminating the reaction using a reference wavelength of 620nm. Reagents and serum samples were dispensed into the wells using a micropipette.

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Table 3.1: Arrangement of wells in the microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank											
B	Blank											
C	Negative Control											
D	Negative Control											
E	Cut-off Control											
F	Cut-off Control.											
G	Positive Control.											
H	Positive Control.											

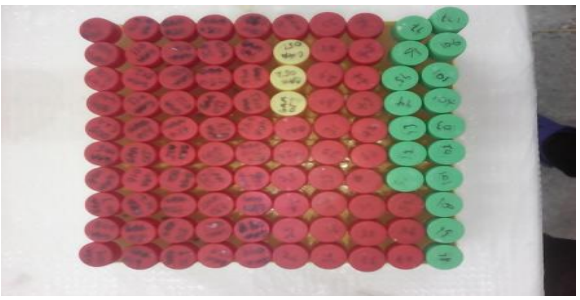

Figure 3.1: Thawing of Samples

Figure 3.2: Sample reading with automated microtitre plate reader

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3.7 Calculation and Interpretation of Results (IgM and IgG)

3.7.1 Calculation of Cut-off (IgM and IgG)

The Cut-off was calculated as the mean absorbance value of the Cut-off Controls determined (Table 3.1).

$$\text{Cut-off} = \frac{\text{absorbance value cut-off control + absorbance value cut-off control}}{2}$$

3.7.2 Calculation of Results in Abcam Units (AU) (IgM and IgG)

$$\text{Results in Abcam Units (AU)} = \frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}}$$

3.7.3 Interpretation of Results (IgM and IgG)

Tests results were interpretated according to Abcam anti-Zika virus Igm and IgG protocols. Interpretation was done according to the table below;

Table 3.2: Criteria for Interpretation of Results

Result	Value
Cut-off	10 AU
Positive	>11 AU
Equivocal	9-11 AU
Negative	<9 AU

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3.8 Independent and Outcome variables

3.8.1 Independent variable

The independent variables were: age, yellow fever vaccination and sex.

3.8.2 Outcome variable

The outcome variable were seropositivity, muscle joint pains, conjunctivitis, diarrhoea, nausea, vomiting, muscle pain, joint pain, jaundice, loss of appetite, chest pain, rapid respiration and recent loss of hearing.

3.9 Statistical Analysis

Clinical and laboratory data were collected and recorded manually. Data were double entered, cleaned and stored in Microsoft Excel. Analyses were done using IBM SPSS version 21 (IBM Corp., Armonk, United States). Bivariate logistic regression model was used to investigate association between Zika virus seropositivity and age, yellow fever vaccination and sex. It was also used to investigate the association between Zika virus seropositivity and clinical characteristics such as muscle joint pains, conjunctivitis, diarrhoea, nausea etc. In the bivariate analysis, variables that were associated with Zika virus seropositivity at a significance level of p-value < 0.05 were considered to be statistically significant. One way-Anova was also used to test the association within groups at a significance level of p-value < 0.05.

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CHAPTER FOUR

RESULTS

4.1.0 Demographic Characteristics of Febrile Patients

A total of 160 febrile patients were enrolled, 119 (74.4%) were females while 41 (25.6%) were males. Eighty seven (54.4%) were within the reproductive age group but four (12.1%) were children. Sixty one (38.1%) were vaccinated against yellow fever whereas 99 (61.9%) were unvaccinated. Demographic characteristics of febrile patients involved in the study are summarized in Table 4.1 below.

Table 4.1: Demographic characteristics of febrile patients

Variable	Number	Percentage (%)
Age (years)		
Children	16	10
Reproductive age	87	54.4
> (above)	57	35.6
Gender		
Male	41	25.6
Female	119	74.4
Yellow fever vaccination		
Vaccinated	61	38.1
Unvaccinated	99	61.9

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4.1.1 Seroprevalence of Zika Virus

Seroprevalence of Zika virus from the study was 20.6%. Of the 33 anti-Zika virus antibodies detected, 3 were anti-Zika virus IgG while 30 were anti-Zika virus IgM. No patient had both anti-Zika virus IgG and IgM antibodies (Figure 4.1).

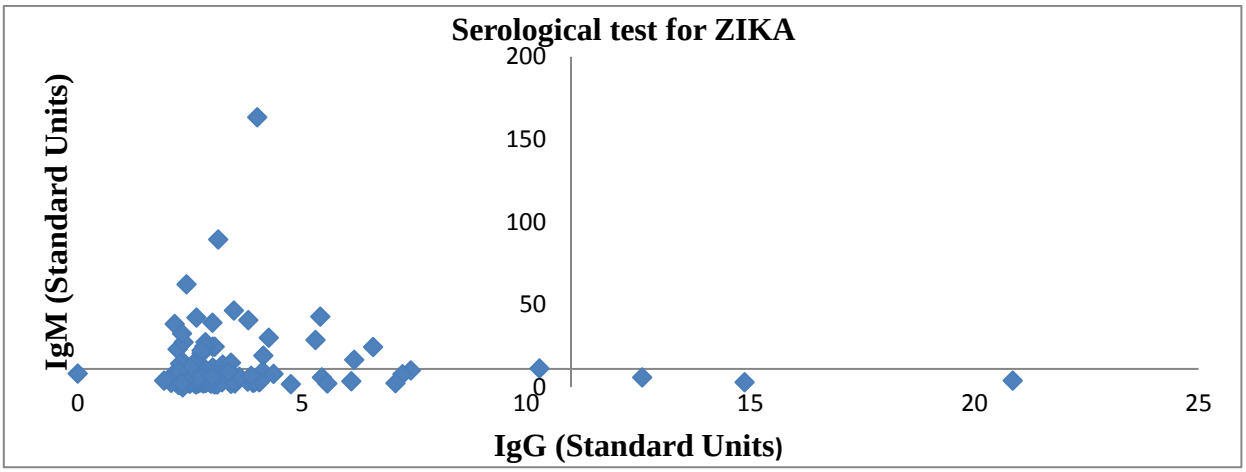

Figure 4.1: Seroprevalence of Zika virus

4.1.2 Characteristics of Zika Virus Seropositives

Among the age groups identified, 14 (42.5%) of the anti-Zika virus IgM were recorded in the reproductive age group but 4 (12.1%) were recorded in children. Anti-Zika virus IgG was detected in two individuals (66.7%) within the reproductive age group and a child 1 (33.4%).

From the total Zika virus seropositives, 11 (33.3%) were males and 22 (66.7%) females. As a result, Zika virus infection was estimated to affect males and females in a ratio of 1:2. Number of anti-Zika virus IgM males was 11 (36.7%) and that of females was 19 (63.3%). Anti-Zika virus IgG was detected only in females representing 100%. No significant

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difference was observed between anti-Zika virus IgM males and females (P=0.016) and IgG males and females (P=0.500).

Twenty (66.7%) and ten (33.3%) of the anti-Zika virus IgM were detected in yellow fever unvaccinated and vaccinated febrile patients respectively with a significant difference of P=0.000. Again, 2 (66.7%) of the anti-Zika virus IgG were detected in unvaccinated febrile patients. Vaccinated febrile patients recorded only one (33.3%) as shown in Table 4.2.

Table 4.2: Characteristics of Zika virus seropositives

Variables	**Zika virus antibodies n= 33**			
	IgM (30)	***P-value***	**IgG (3)**	***P-value***
Age groups				
Children	4(12.1%)		0	
Reproductive age	14(42.5%)	0.08	2(66.7%)	-
> (above)	12(36.4%)		1	
Gender				
Male	11(36.7%)		0	
Female	19(63.3%)	0.016	3(100%)	-
Yellow fever vaccination				
Vaccinated	10(33.3%)		1(33.3%)	
Unvaccinated	20(66.7%)	0.000*	2(66.7%)	-

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One –way Anova was used to estimate the difference between groups. "n" represents the number of Zika virus seropositives. * denotes statistically significant difference between groups. P is significant at 0.05

4.1.3 Monthly Distribution of Anti-Zika Virus Antibodies

Anti-Zika virus IgM detection peaked in March 7 (23.3%) and May 9 (30%) and decreased in September 1 (3.3%). However, anti-Zika virus IgG were detected only in January and March, 2 (66.7%) and 1 (33.3%) respectively. Anti-Zika virus IgM monthly distribution showed no significant difference (P = 0.06). Again, no significant difference was observed between anti-Zika virus IgG monthly distribution (P=0.205) (Figure 4.2).

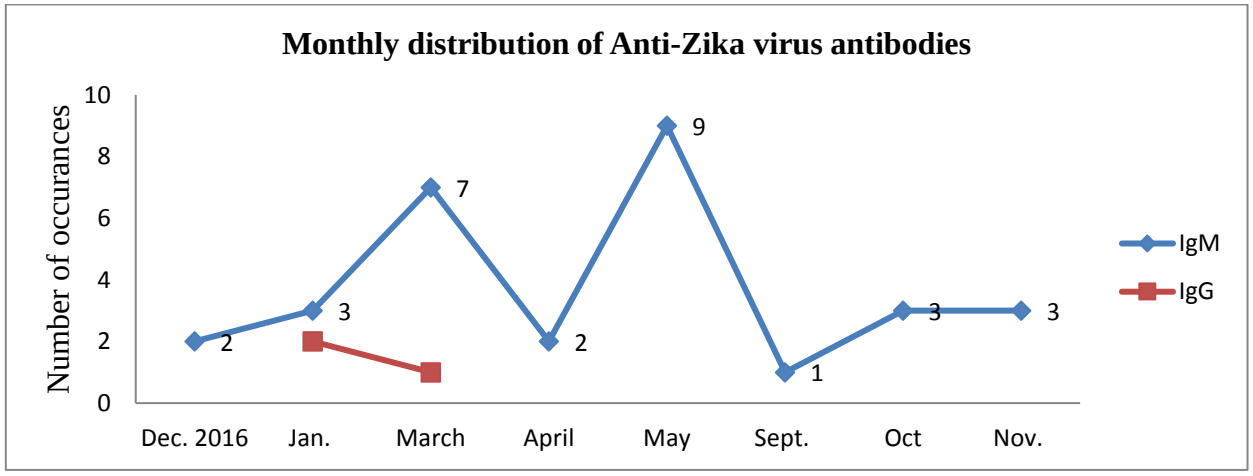

Figure 4.2: Monthly distribution of anti-Zika virus antibodies

4.1.4 Anti-Zika Virus IgM Monthly Distribution Stratified by Gender

Anti-Zika virus IgM females were recorded in the month of May 8 (24.2%) whereas, males were recorded in the month of March 3 (9%) (Figure 4.3)

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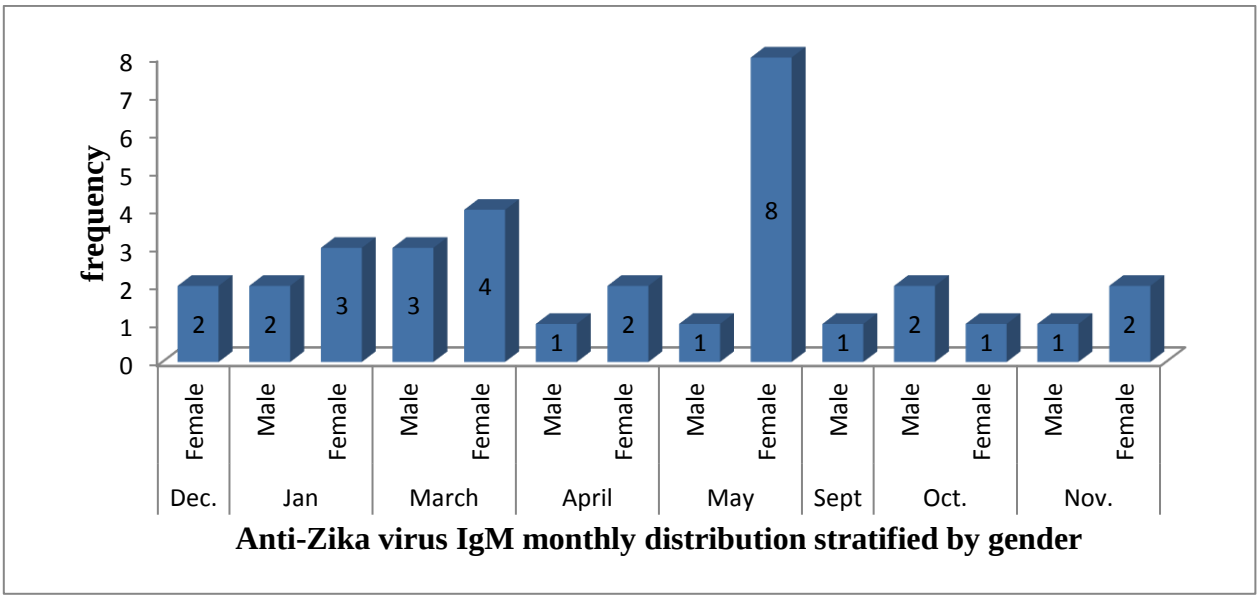

Figure 4.3: Anti-Zika virus IgM monthly distribution stratified by gender

4.1.5 Date of Anti-Zika virus IgM detection

The study recorded 8 (24%) of the anti-Zika virus IgM on the 2nd day after the onset of illness. One (3%) of the anti-Zika virus IgM was detected on the 7th and 8th days respectively (Figure 4.4).

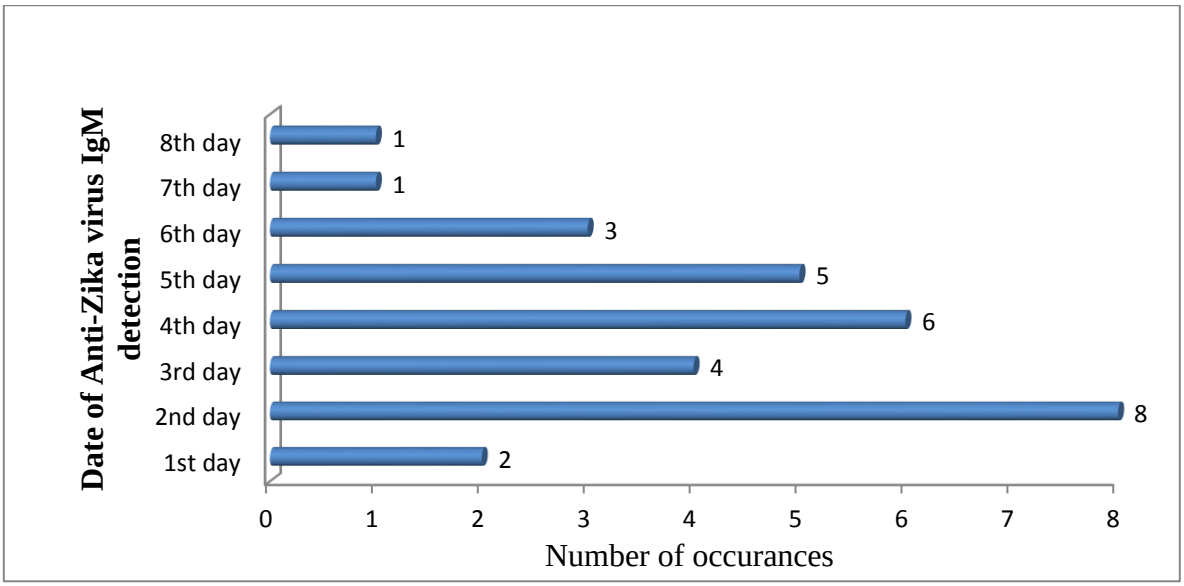

Figure 4.4: Date of Anti-Zika virus IgM detection

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4.1.6 Town of Residence of Zika Virus Seropositive Patients

Proportion of Zika virus seropositives residing in Lapaz and Amasaman accounted for 4 (12.1%) each. Ashaley Botwe, Adabraka, Adenta and Kosoa recorded 2 (6.1%) each. One (3.0%) each was recorded in 37, Alajo, Asylum down, Ordorna, Dome kwabenya, Teshie, Airport, Darkuman, Nima, Weija, Pokuase, Kokomlemle, Winneba, Achimota, Circle, Awodome and Asafo.

4.1.7 Predictors of Zika Virus Seropositivity

Bivariate logistic regression analysis revealed that gender, age and yellow fever vaccination were not associated with Zika virus seropositivity (Table 4.3).

Table 4.3: Bivariate logistic regression analysis showing predictors of Zika virus seropositivity

Variables	OR (95% Cl)	*P-value*
Age groups		
3-20	1	
21-30	0.293 (0.048-1801)	0.185
31-40	0.480 (0.094-2.458)	0.378
41-50	0.778 (0.141-4.304)	0.773
51-60	0.283 (0.52-1.548)	0.145
61 and above	0.533 (0.076-3.725)	0.526
Sex		

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Male	1	
Female	0.696 (0.306-1.587)	0.389
Vaccination	0.935 (0.426-2.049)	0.866

4.2 Symptoms Presented by Seropositive Febrile patients

From the figure below, 33 (100%) of the seropositive febrile patients were presented with fever. The least 1 (0.8%) symptom reported was Jaundice. Conjunctivitis was not reported by any seropositive Zika virus female. All other symptoms were reported in both males and females.

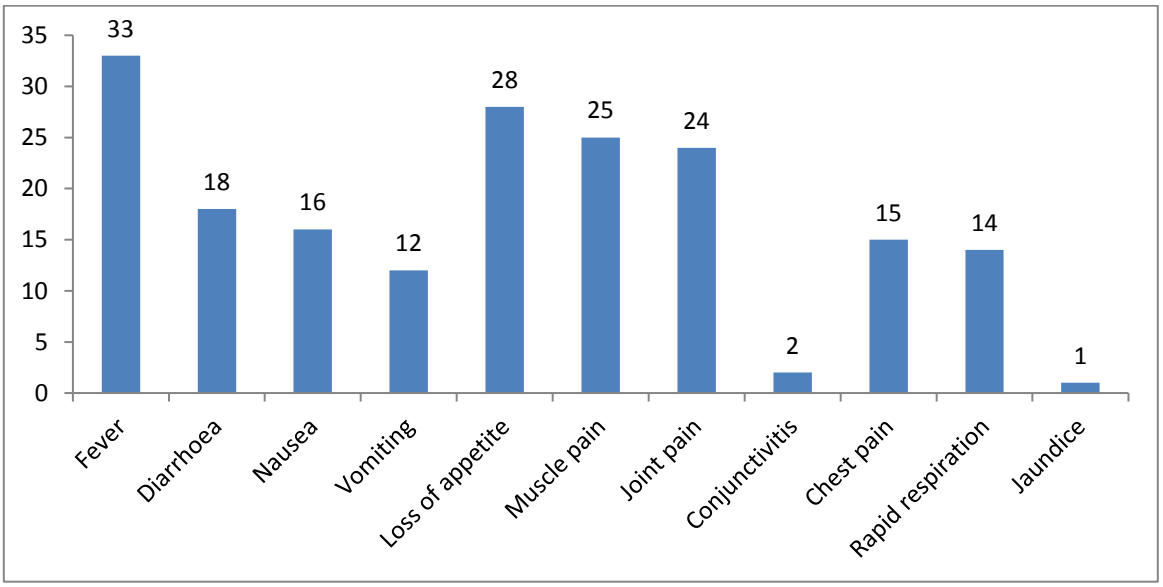

Figure 4.5: Symptoms presented by seropositive febrile patients

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4.2.1 Bivariate Analysis of Zika Virus Seropositive and Clinical Presentation

Zika virus seroprevalence was associated with muscle pain, joint pain, conjunctivitis and chest pain as shown in the bivariate logistic regression analysis table below (Table 4.5).

Table 4.4: Bivariate logistic regression analysis

Variables	**OR (95% Cl)**	***P-value***
	n=33	
Chest pain	-	0.000*
Fever	0.686(0.256-1.838)	0.453
Diarrhoea	0.000(0.000)	0.999
Nausea	0.476(0.178-1.277)	0.140
Loss of appetite	1 (0.367-2.727)	1.000
Muscle pain	0.102(0.31-0.334)	0.000*
Joint pain	0.183(0.063-0.531)	0.002*
Conjunctivitis	0.214(0.075-0.609)	0.004*
Rapid Respiration	9.143(1.855-45.056)	0.007
Vomiting	0.607(0.227-1.625)	0.320

"n" represents the number of Zika virus seropositive .* denotes statistically significant difference. P is significant at 0.05

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CHAPTER FIVE

DISCUSSION, CONCLUSION, RECOMMENDATION AND LIMITATION

5.1 Discussion

Data from the study indicates exposure to Zika virus which suggests the possible circulation of the virus in Ghana. The recent rapid expansion, association with severe neurological disorders and the overlapping clinical features of Zika virus infection with other endemic disease conditions have made prediction of exposed population a necessity. Until recently, identifying exposed population has been of a low priority because of the mild symptoms presented by Zika virus infection (Petersen *et al.,* 2016). This study therefore sought to bring some degree of attention to Zika virus infection in Ghana where little is been done on surveillance. It was conducted to serologically determine and establish Zika virus infection in febrile patients in a selected health facility, the Greater Accra regional hospital in Accra, Ghana.

Cross-reactive antibodies produced by flaviviruses have made diagnosis of Zika virus infection difficult especially, when dengue has been found in the sub-region. A study conducted by Safronetz *et al.,* (2017**)** to evaluate the performances of 5 commonly used Zika virus immunoassays against an ELISA (MAC ELISA) developed by the Center for Disease Control and Prevention and cross-PRNTs showed that, the Abcam ELISA kits used in this study has a specificity of 100% and a sensitivity of 57% (Safronetz *et al.,* 2017).

Zika virus infection (33 seropositive febrile patients) was not widespread among the febrile patients. The prevalence of Zika virus antibodies recorded from the study, 20.6% was in line with findings from other African countries, 1.3-52% prevalence level (Akoua-Koffi *et*

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al., 2001). However, prevalence recorded was greater than seroprevalence in hospital admissions in Zambia 6%, Senegal 0.1%, Gabon 0.1%, Ethiopia 10% but less than Cameroon 38%. Prevalence was also higher than seroprevalence in the French Polynesia 0.8% before the epidemic based on screening banked blood from 593 blood donores (Aubry *et al.,* 2015). These differences could be due to inconsistency in the study participants' inclusion criteria or diagnostic test used (Rupert *et al.,* 2014). This study recruited febrile patients whereas those studies were conducted in the general population.

Despite the low seroprevalence of Zika virus recorded, real-time reverse transcription polymerase chain reaction (RT-PCR) and plaque reduction neutralization test (PRNT) which could eliminate the potential for cross-reactivity and increase sensitivity were not used in this study.

Of the 160 febrile patients, 3 (1.9%) tested positive for anti-Zika virus IgG, suggestive of previous exposure to Zika virus (Vaughn *et al.*, 2000). This indicates susceptibility level of 98.1% among the study population which confirms studies carried out in other sub-Saharan African countries (Cannon *et al.,* 2010). Due to the high susceptibility levels (98.1%), appropriate preventive measures should be taken to curb the occurrence of congenital infection and devastating consequences of Zika virus infection.

These preventive measures as advised by Matthew *et al.,* (2016) include practicing mosquito abatement, employing mosquito bite avoidance behaviors and taking precautions to reduce the risk for sexual transmission. Thirty (18.8%) patients had anti-Zika virus IgM. The observed IgM prevalence could be due to recent exposure to Zika virus or a wave of Zika virus infection. No patient had both anti-Zika virus IgG and IgM. The absence of

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patients with both anti-Zika virus IgM and IgG show no possible recurrent Zika virus infection (Lazzarotto *et al.,* 2008).

Among the age groups identified, 14 (42.5%) of the anti-Zika virus IgM were recorded in the reproductive age group but 4 (12.1%) were recorded in children, with no significant difference of P = 0.08. This may be due to the pattern of sexual activities and exposure among the age groups. Active sexual involvement and exposure to Zika virus has been proven to begin in the reproductive age group, increases gradually and then declines as one ages (Ayensu *et al.,* 2014). Taking into account the possible adverse effects associated with Zika virus infection, it is important to focus on prevention efforts in this age group and surveillance.

Anti-Zika virus IgG was detected in two individuals (66.7%) within the reproductive age group and a child 1 (33.4%). This data suggests that, Zika virus infection is not endemic in Ghana, rather the virus had been introduced to a non-exposed population as endemicity is attained when the adult infection decreases and only the new entrants into the population are affected more (Kumar *et al.,* 2015). This is different from what has been observed around the world in feberile patients (Fatim *et al.,* 2000).

High prevalence of anti-Zika virus IgM or IgG in the reproductive age group compared to children supports data from earlier studies by Matthew *et al.,* (2016) and Kumar *et al.,* (2015). Possible explanation to this observation could be the activeness of the reproductive age group during the early hours of the day which exposes them to the bites of Zika virus-carrying *Aedes* spp. of mosquitoes more frequently than children (World Health Organization, 2009).

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Zika virus infection was estimated to affect females and males in a ratio of 2:1 which correlate well with several studies (Mathew *et al.*, 2016; Plourde and Bloch, 2016). Number of anti-Zika virus IgM males was 11 (36.7%) and females, 19 (63.3%). Female accounting for greater number of anti-Zika virus IgM is consistent with a study conducted in Puerto Rico in 2016 which showed females to be more predisposed to Zika virus infection (Matthew *et al.*, 2016).

Considering the findings of Matthew *et al.*, (2016) and that of this study, there may be gender-related differences in Zika virus infection incidence, which might be due to exposure differences (Kaplan *et al.*, 1983). Activeness of females during the day time exposes greater proportion of females to Zika virus-carrying *Aedes* spp. of mosquitoes either at work or while travelling to and from work (Matthew *et al.*, 2016). This may also be attributed to possible differences in who sought medical care following symptomatic infection (Matthew *et al.*, 2016).

Yew *et al.* found males to be significantly more likely to have past Zika virus infection in a multivariate regression analysis (Yew *et al.*, 2009). In contrast, this study demonstrated females to have past Zika virus infection, anti-Zika virus IgG of 100% (3). But no significant difference was observed between anti-Zika virus IgM males and females (P=0.166) and IgG males and females (P=0.500). Absence of anti-Zika virus IgG in males could mean that males are highly susceptible to Zika virus infection.

Analysis of data based on yellow fever vaccination revealed high prevalence 20 (66.7%) of anti-Zika virus IgM in yellow fever unvaccinated compared to yellow fever vaccinated individuals 10 (33.3%) with a significant difference of P=0.000. Further, one (33.3%) anti-

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Zika virus IgG was recorded in yellow fever vaccinated and two (66.7%) in unvaccinated. Finding agrees with Rupert *et al.,* (2014) and Stoler *et al.,* (2015), and may suggests that yellow fever vaccination could provide immunity against Zika virus infection probably due to the cross-reactive antibodies produced by yellow fever vaccines (Cavalcanti *et al.,* 2016).

This draws parallel with a research in Brazil by Cavalcanti *et al.,* (2016). However, in a bivariate logistic regression analysis, yellow fever vaccination was not associated with Zika virus seropositivity (P>0.05). This was in contrast to the results of Bouba *et al.,* (2017); in a multivariate analysis, yellow fever vaccination was significantly associated with Zika virus seropositivity. Bivariate logistic regression analysis revealed that gender and age were not associated with Zika virus seropositivity. This observation is in accordance with a prevalence study of symptomatic Zika virus infection by age and gender in Puerto Rico, 2016 (Matthew *et al.,* 2016).

Zika virus antibodies detection has been found to vary by season. Detection peaks in the raining season and decreases in the dry season (Heang *et al.,* 2012; Heang *et al.,* 2011). Similar to Mark *et al.,* (2009), monthly trends indicate that anti-Zika virus IgM detection peaked in March 7 (24.2%) and May 9 (30%) and decreased in September 1 (3.3%). Interestingly, this was different for anti-Zika virus IgG. Anti-Zika virus IgG peaked in January and decreased in March representing 2 (66.7%) and 1 (33.3%) respectively. Anti-Zika virus IgM monthly detection showed no significant difference (P = 0.006). No significant difference was observed between anti-Zika virus IgG monthly detection (P=0.205).

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Monthly distribution of Zika virus infection with higher prevelence in the raining season as seen in this study synchronizes with reported pattern of Zika virus transmission (Gupta *et al.*, 2006). The high frequency of anti-Zika virus IgM in the months of March and May could be due to the high amount of rainfall which provided temperatures suitable for virus survival, incubation, development and biting of *Aedes aegypti* and *Aedes albopictus* (Mordecai *et al.*, 2016). The compact clusters of cases in March and May, and the high prevalence of IgM against Zika virus are consistent with an acute outbreak of Zika virus infection in the population without previous immunity to Zika virus (Mark *et al.*, 2009).

In the absence of data on the estimated persistence of anti-Zika virus IgM and reports of outbreak of Zika virus infection, but anti-Zika virus IgM can be detected after 90 days of infection (Nogueira *et al.*, 1992); outbreak might have resulted from a recent contact with Zika virus. Monthly distribution of Zika virus infection is very important at the local level for effective control measures. Preventive measures to curb the occurrence of any congenital anomalies associated with Zika virus infection should come into full action during water stagnation periods after the initial bouts of rainfall (Ukey *et al.*, 2010 and Kumar *et al.*, 2015).

Relatively, majority (6) of the anti-Zika virus IgM females were recorded in the month of May while 3 of anti-Zika virus IgM males were recorded in the month of March. Yew *et al.* suggested that, dissimilarities in the type of work might account for the differences in number of female to male in the monthly distribution of Zika virus antibodies (Yew *et al.*, 2009). Again, reporting biases by health care workers, severity of symptoms among gender

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(male and female), sexual transmission and differences in health care seeking behavior may also account for the differences in the distribution of Zika virus antibodies (Dos Santos *et al.*, 2016).

Sera from patients who reside at Lapaz and Amasaman recorded the highest seropositivity of 12.1% each. Ashaley Botwe, Adabraka, Adenta and Kosoa recorded 2 (6.1%) each. One (3.0%) was recorded in 37, Alajo, Asylum down, Ordorna, Dome kwabenya, Teshie, Airport, Darkuman, Nima, Weija, Pokuase, Kokomlemle, Winneba, Achimota, Circle, Awodome and Asafo. The high number of Zika virus seropositive patients residing in Abeka Lapaz and Amasaman could be due to environmental factors present in these localities which favour the breeding of the active vector *Aedes* spp. of mosquitoes. These environmental factors according to Thomson *et al.*, (2006) include precipitation and temperature. Additionally, housing type and lifestyle present in these localities may also contribute to populations' contact with the active vector *Aedes* spp. of mosquitoes (Moreno-Madrin˜´an and Turell, 2017).

The study recorded 8 (24%) of the anti-Zika virus IgM on the 2nd day after the onset of illness. One (3%) of the anti-Zika virus IgM was detected on the 7th and 8th days respectively. This indicates anti-Zika virus IgM is detectable in high numbers on the first day of infection to the fifth day but persists in low numbers after the fifth day. This observation is consistent with what has been observed in other countries (Sant´e, 2015). This further suggests the seropositive febrile patients to be recently infected since anti-Zika virus IgM are detectable within two to seven days after infection (Vaughn *et al.*, 2000).

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Eighty percent of Zika virus infections are asymptomatic (Shan *et al.*, 2016). Symptomatic cases account for about 18-20%, manifest with nonspecific clinical symptoms and resemble dengue or chikungunya confounding diagnosis (Duffy *et al.*, 2009). In this study, commonly reported symptoms of Zika virus infection documented were fever, muscle pain, joint pain and conjunctivitis. This is similar to a Zika virus infection clinical profile documented in Martinique in asymptomatic blood donors (Gallian *et al.*, 2016). Strikingly, conjunctivitis often observed in Zika virus infection (Heukelbach *et al.*, 2016), was not reported by any seropositive female. This agrees with a study conducted in Brazil by Cassia *et al.*, (2016). Digestive symptoms noted in Zika virus infected patients but rarely observed, however, found in this study include nausea, vomiting and diarrhea (Oehler *et al.*, 2013). A bivariate logistic regression analysis demonstrated an association between Zika virus seropositivity and chest pain, conjunctivitis, muscle pain and joint pain ($P<0.05$).

5.2 Conclusion

Results from this study indicate the possible exposure of these febrile patients to Zika virus with a prevalence of 20.6%. This may be the tip of the iceberg since our population size is relatively small compared to the total number of febrile patients in Ghana. Characteristics such as age, gender and yellow fever vaccination were found not to be the predictors for Zika virus infection.

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5.3 Recommendations

The current study revealed possible exposure of these febrile patients to Zika virus and therefore this study should be replicated in other parts of Ghana to determine the overall susceptibility levels in the entire country.

Again, a critical study in newborns would be necessary to estimate the number of newborns who suffer abnormalities because of maternal primary infection among the few susceptible individuals. This is due to overlapping symptoms of most of the childhood illnesses.

Lastly, screening of febrile patients form health centres of communities identified with ant-Zika virus antibodies.

5.4 Limitations

Despite the low seroprevalence of Zika virus recorded, real-time reverse transcription polymerase chain reaction (RT-PCR) and plaque reduction neutralization test (PRNT) which could eliminate the potential for cross-reactivity and increase sensitivity were not used in this study.

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APPENDIX

Appendix 1: Case Investigation Form

NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH (NMIMR)
COLLEGE OF HEALTH SCIENCES, UNIVERSITY OF GHANA, LEGON

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Appendix I

CASE INVESTIGATION FORM

Molecular Epidemiology of Dengue Fever and Chikungunya Viruses in Ghana

Case identification number: ..... Urban Rural

Identification
District: ..... Town: .....

Nearest Health Facility: .....

Name of Patient: .....

Father/Mother (if applicable): .....

Date of Birth/Age: ..... Sex.....

Notification/Investigation

Date of notification: ...../...../..... Notified by: .....

Date investigated: .....

History

List districts patient visited last 2 weeks

Fever of ≥38°C: Yes/No ...../..... /..... 1.....2.....
Date of first appearance

Jaundice: Yes/No...../..... /.....
Date of first appearance

Haemorrhage: Yes/No ...../..... /..... Were there other cases of jaundice
Date of first appearance in this district or other districts? Yes /No

Date of last YF Vaccination ...../..... /..... ...../..... /.....
Date of first appearance

Blood Specimen

Date 1st specimen: ...../...../..... Date 2nd specimen: ...../..... /.....

NMIMR-IRB Form A (NMIMR Researchers Only)
Version Date: May 2013

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Date processed: ……/……../…… Date processed: ……. /……../……..

Other clinical specimen taken (please specify) ……..

Test Results
Yellow fever virus: IgM/IgG antibody test result: ……..
Dengue fever virus types 1-4 IgM/IgG antibody test result: ……..
Chikungunya virus IgM/IgG antibody test result: ……..
Other arboviral infection IgM test (specify): ……
PCR test result: ……
Date results sent to surveillance unit /clinician: ……../……../……

Final classification of case: ……

Send copy of this completed form immediately to: National Disease Surveillance Department. Korle Bu, Accra. Tel.: 021-663606
For further information, please contact Mr. Michael Adjabeng, Tel.: 0208157618
Virology Dept. NMIMR, Attn: Dr Kofi Bonney, Tel.: 0243 568 041

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APPENDIX II

Symptoms(Check each if present)	Yes	No	Date of appearance
Fever ≥ 38°C			
Diarrhoea			
Extreme weakness after rehydration			
Nausea			
Vomiting			
Loss of appetite			
Muscle pain			
Joint pain			
Conjunctivitis			
Chest pain			
Rapid respiration			
Recent loss of hearing			
Bleeding, specify below:			**Date of first appearance**
Black or bloody vomits			
Black or bloody stool			
Mouth			
Nose			
Bloody urine			
Skin or puncture site			
Other bleeding, specify			
Other observations, specify			

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Appendix 2: Characteristics of Patients

Town of Residence	Age	Sex	Date of onset	Date of last YF vaccination	Diar.	Nau.	Vom.	Lap.	Mp	Jp	Conj.	Cp	Rr
Ablekuma	66	M	16/09/2017	No	N	Y	Y	Y	Y	Y	N	Y	Y
Weija	36	F	14/09/2017	No	Y	Y	N	Y	Y	Y	N	N	N
Kwashieman	26	F	15/09/2017	2011	Y	Y	Y	N	Y	Y	N	N	
Pokuase	26	F	16/09/2017	2011	N	N	N	Y	Y	Y	N	N	N
Lapaz	64	F		No	N	N	N	N	Y	Y	N	Y	N
Gbawe	29	F		No	Y	N	N	Y	N	N	Y	N	N
Ashiaman	31	F	19/09/2017	No	N	N	N	N	N	Y	N	Y	N
Abeka Lapaz	43	F		No	N	N	N	N	Y	N	N	N	N
Sakumono	79	F		No	N	N	N	N	Y	Y	N	Y	N
Awoshie	30	F	24/09/2017	No	N	N	N	Y	Y	Y	N	Y	
Kokomlemle	52	M	23/09/2017	No	N	N	N	Y	Y	Y	N	Y	N
Kasoa	32	F	23/09/2017	2011	Y	Y	N	Y	Y	Y	N	Y	Y
Kokomlemle	20	F	22/09/2017	No	N	N	N	Y	Y	Y	N	Y	Y
Lapaz	27	F	21/09/2017	2011	N	Y	Y	Y	Y	Y	N	Y	N
Nima	24	M	23/09/2017	No	Y	Y	N	Y	Y	Y	N	N	N
Winneba	47	M	25/09/2017	No	N	N	N	Y	Y	Y	N	N	Y
Achimota	32	F	22/09/2017	2011	Y	Y	N	Y	Y	Y	N	N	N
Awoshie	28	F	29/09/2017	No	N	Y	Y	Y	Y	Y	N	N	N
	25			No									
Kasoa	14	f	08/10/2017	No	Y	N	Y	Y	Y	N	N	N	Y
Achimot	52	M	09/10	No	Y	Y	N	Y	Y	Y	N	Y	N

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a			/2017										
Kaneshie		F		No	N	Y	N	Y	Y	Y	N	Y	Y
Circle	58	M	04/10/2017	No	N	Y	Y	Y	Y	Y	N	N	Y
Adabraka	67	F	06/10/2017	No	Y	Y	Y	Y	Y	Y	N	Y	Y
Ridge-Accra	44	F	08/10/2017	No	Y	Y	Y	Y	Y	Y	N	Y	N
Mallam	30	M	19/10/2017	No	Y	N	N	N	Y	N	N	N	N
Teshie		F	20/10/2017	No	N	N	N	N	Y	N	N	Y	N
Amasaman	38	M	19/10/2017	No	N	N	N	N	N	N	N	N	N
West Legon	46	F	21/10/2017	No	N	N	N	Y	Y	N	N	N	N
Nsawam	32	F	20/10/2017	No	N	Y	N	N	N	N	N	N	N
McCarthy Hill	48	F	22/10/2017	No	N	N	N	Y	N	N	N	N	N
Lapaz Nii Boye Town	37	F	18/10/2017	No	N	N	N	N	N	N	N	N	N
Amasaman	62	F	29/10/2017	No	N	N	N	N	Y	N	N	N	N
Madina	31	F	26/10/2017	No	N	N	N	Y	N	N	N	N	N
Asheley Botwe	39	F	23/10/2017	No	N	N	N	N	N	N	N	N	N
Akweteyman	49	F	27/10/2017	No	N	Y	N	N	N	N	N	N	N
Kokomlemle	58	F	25/10/2017	No	N	N	N	Y	Y	N	N	N	N
Adenta	31	M	27/10/2017	No	Y	N	N	Y	N	N	N	N	N
Amasaman	49	F	28/10/2017	No	N	N	N	Y	N	N	N	N	N
Osu	71	F	28/10/2017	No	N	Y	Y	N	Y	N	N	N	N
Kotobabi	35	F	02/11/2017	No	Y	N	N	N	N	N	N	Y	Y
Bubuashie	39	F	03/11/2017	No	N	N	N	N	N	N	N	N	N
Ridge	53	M	05/11/2017	No	N	N	N	Y	N	N	N	N	N

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Achimota	80	F	05/11/2017	No	N	N	N	N	Y	Y	N	N	N
Osu	61	F	09/11/2017	No	N	N	N	Y	Y	N	N	N	N
Amasaman	35	F	09/11/2017	No	N	N	N	N	N	N	N	N	N
Madina	45	M	10/11/2017	No	N	Y	N	N	N	N	N	N	N
Kanda	35	M	08/11/2017	No	N	N	N	N	N	N	N	N	N
Dome	60	M	16/11/2017	No	N	N	N	N	Y	N	N	N	N
Awodome	3	F	18/11/2017	No	N	N	N	N	N	N	N	N	N
Amasaman	40	F	17/11/2017	No	N	N	N	Y	N	N	N	N	N
Botwe	23	F	18/11/2017	No	Y	N	N	N	N	N	N	N	N
Asofa	9	M	20/11/2017	No	N	N	N	N	N	N	N	N	N
Pokuase	32	F	17/12/2016	2011	Y	Y	Y	Y	Y	Y	N	Y	Y
Agiriganoo	34	F	17/12/2016	2011	N	N	N	Y	Y	Y	N	Y	
Kaneshie	23	F	16/12/2016	2011									
Anyaa	59	F	16/12/2016	No	N	Y	Y	Y	Y	Y	N	N	N
Abeka	39	F	21/12/2016	No	N	N	N	Y	Y	Y	N	Y	N
Osu	15	M		2011	Y	N	N	Y	Y	Y	N	N	N
37	29	F	24/12/2016	2011	Y	N	N	Y	Y	Y	N	Y	Y
	24	F	24/12/2016	2011	Y	Y	Y	Y	N	N	N	Y	Y
Adabraka	61	F	20/12/2016	2012	Y	Y	Y	Y	N	N	N	N	Y
Spintex	31	F	21/12/2016	No	Y	N	N	Y	Y	Y	N	Y	Y
Mesie	42	F	09/01/2017	No	Y	Y	Y	Y	Y	Y	N	Y	Y
Kokomlemle	44	F	09/01/2017	No	Y	Y	Y	Y	Y	Y	N	Y	Y
East Legon	22	F	08/01/2017	No	Y	N	N	Y	Y	Y	N	Y	Y
Asylum	22	F	09/01	No	Y	N	N	Y	Y	Y	N	Y	Y

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Down			/2017										
Nungua	27	F	10/01/2017	No	Y	Y	Y	Y	N	Y	N	Y	Y
Spintex	38	F	07/01/2017	2011	Y	Y	Y	Y	Y	Y	N	N	Y
Achimota	9	M	10/01/2017	2008	Y	Y	Y	Y	Y	Y	N	N	N
Ridge	30	M	13/1//2017	2010	N	N	N	N	Y	Y	N	Y	Y
Botwe	60	F	10/01/2017	No	N	Y	Y	Y	Y	Y	N	N	Y
Spintex	44	F	14/01/2017	No	N	N	N	N	N	Y	N	N	N
Madina	49	M	09/01/2017	No	Y	N	N	Y	N	N	N	N	N
Adabraka	24	M	11/01/2017	No	N	Y	Y	Y	Y	Y	N	Y	Y
Sowutuom	48	F	08/01/2017	No	Y	Y	Y	Y	Y	Y	N	N	N
Ordorna	21	F	10/01/2017	2010	N	N	N	Y	Y	Y	N	Y	Y
Accra Newtown	27	M	11/01/2017	2010	Y	Y	Y	Y	Y	Y	N	Y	Y
Nungua	11	F	14/01/2017	2009	Y	Y	Y	Y	Y	Y	N	N	N
Achimota	45	M	12/01/2017	2010	N	Y	Y	Y	Y	Y	N	Y	Y
Lapaz	25	F	12/01/2017	No	Y	Y	Y	Y	Y	Y	N	Y	N
Kasoa	16	M	20/01/2017	No									
Nsawam	63	M	16/03/2017	No	N	N	N	Y	Y	Y	N	Y	N
Nima	54	F	19/03/2017	No	N	N	N	Y	Y	Y	N	N	Y
Madina	34	F	20/03/2017	2010	Y	Y	N	Y	Y	Y	N	Y	Y
Adabraka	20	F	14/03/2017	2010	Y	N	N	Y	Y	Y	N	Y	Y
Weija	29	M	18/03/2017	2010	Y	Y	Y	N	Y	Y	N	N	N
Kasoa	87	M	15/03/2017	No	Y	Y	Y	Y	Y	Y	N	N	Y
Pokuase	25	F	18/03/2017	2010	Y	Y	Y	Y	Y	Y	N	Y	Y

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Lapaz	29	F	18/03/2017	2010	Y	Y	Y	Y	N	N	N	Y	Y
Tema	30	F	18/03/2017	2010	N	Y	Y	Y	Y	Y	N	Y	Y
Lapaz	28	F		2010	Y	N	N	Y	Y	Y	N	Y	Y
Korle-Bu	22	F	20/03/2017	2010	Y	N	N	Y	Y	Y	N	N	N
Accra Newtown	32	F	20/03/2017	2010	Y	Y	N	Y	Y	Y	N	Y	Y
Lapaz	78	F	19/03/2017	No	N	Y	Y	Y	Y	Y	N	N	N
Kokomlemle	21	F	20/03/2017	No	N	N	N	Y	Y	Y	N	Y	Y
Achimota	34	F	16/03/2017	No	Y	N	N	Y	N	N	N	Y	Y
Osu	25	F	21/03/2017	2010	N	N	N	Y	Y	Y	N	N	N
Teshie	44	F	26/03/2016	No	Y	Y	Y	Y	Y	Y	N	Y	Y
Newtown	17	F	19/03/2017	2010	N	N	N	Y	Y	Y	N	N	N
Kasoa	37	M	20/03/2017	2010	Y	Y	N	Y	Y	Y	N	N	Y
Nima	67	M		2010	N	N	N	Y	Y	Y	N	N	N
Amasaman	32	M	23/03/2017	2010	N	Y	Y	Y	Y	Y	N	Y	Y
Madina	32	F	24/03/2017	No	Y	Y	Y	Y	Y	Y	N	Y	N
Madina	40	F	25/03/2017	No	N	N	N	Y	Y	Y	N	Y	N
Weija	26	F	25/03/2017	2010	Y	Y	N	Y	Y	Y	N	N	Y
Madina	33	F		2010	N	Y	N	Y	Y	Y	N	Y	N
Sapeiman	34	F	20/03/2017	No	N	Y	N	Y	Y	Y	N	Y	Y
Adabraka	51	M	21/03/2017	No	N	N	N	Y	Y	Y	Y	N	Y
Kanda	29	F	22/03/2017	No	N	N	N	Y	Y	Y	N	Y	Y
Kasoa	44	F	21/03/2017	No	Y	Y	N	Y	Y	Y	N	Y	Y
Kiseiman	20	F	20/03/2017	2010	N	N	N	Y	Y	Y	N	N	Y
Dome	24	F	19/04	2010	N	N	N	Y	Y	Y	N	Y	Y

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			/2017										
North Kaneshie	37	M	19/04 /2017	2010	Y	Y	N	Y	Y	Y	N	N	Y
Sakaman	23	F	21/04 /2017	No	N	N	N	Y	Y	Y	N	N	N
Darkuman	23	F	23/04 /2017	2010	Y	N	N	Y	Y	Y	N	Y	Y
Madina	29	F	22/03 /2017	2010	N	N	N	Y	Y	Y	N	Y	Y
Kokomlemle	33	M	22/04 /2017	2010	Y	N	N	Y	Y	Y	N	N	N
Dome Kwabenya	37	F	23/04 /2017	No	N	N	N	Y	Y	Y	N	Y	N
Madina	70	F	20/04 /2017	No	N	Y	Y	Y	Y	Y	N	Y	N
Kanda	68	F	22/04 /2017	No	Y	N	N	Y	Y	Y	N	N	N
Adenta	17	M	24/04 /2017	2010	Y	Y	Y	Y	N	N	N	Y	N
Alajo	29	F	25/04 /2017	2010	Y	Y	Y	Y	Y	Y	N	Y	Y
Pokuase	25	F	27/04 /2017	2010	Y	Y	Y	Y	Y	Y	N	N	Y
Alajo	42	F	30/04 /2017	No	N	Y	N	Y	Y	Y	N	N	N
Asylum Down	38	F	02/05 /2017	2010	Y	N	N	Y	Y	Y	N	N	Y
Kasoa	49	F	01/05 /2017	No	Y	Y	N	Y	Y	Y	N	Y	Y
West Legon	31	F	27/04 /2017	2010	N	Y	N	Y	Y	Y	N	Y	Y
Osu	55	F	04/05 /2017	No	Y	N	N	Y	Y	Y	N	Y	N
Darkuman	42	F	02/05 /2017	No	Y	N	N	Y	Y	Y	N	N	N
Adabraka	42	F	04/05 /2017	2011	Y	N	N	Y	Y	Y	Y	Y	Y
Achimota	33	F	06/05 2017	2011	Y	N	N	Y	Y	Y	N	Y	Y
Madina	51	F	06/05 /2017	No	Y	Y	N	Y	Y	Y	N	Y	Y
Weija	26	M	02/05 /2017	No	Y	Y	Y	Y	Y	Y	N	N	N
Amasaman	30	F	06/05 /2017	2011	Y	N	N	Y	Y	Y	N	N	N

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Lapaz	53	M	02/05/2017	No	N	Y	Y	Y	Y	Y	N	N	N
Circle	23	M	06/05/2017	2011	Y	Y	Y	Y	Y	Y	Y	N	Y
Alajo	13	M	01/05/2017	2005	Y	Y	Y	N	Y	Y	N	Y	N
Achimota	45	F	20/05/2017	2011	Y	Y	Y	Y	Y	Y	N	Y	Y
Mamprobi	63	F	19/05/2017	No	Y	Y	Y	Y	Y	Y	N	Y	Y
Odorkor	30	F	17/05/2017	No	Y	N	N	Y	Y	Y	N	Y	Y
Osu	27	F	20/05/2017	2011	N	Y	N	Y	Y	Y	N	N	N
Airport	20	F	20/05/2017	2010	Y	N	N	Y	Y	Y	N	Y	N
Pig Farm	37	F	20/05/2017	No	N	Y	Y	Y	Y	Y	N	N	N
Dome	37	F	19/05/2017	No	N	Y	Y	Y	Y	Y	N	Y	N
Lapaz	59	F	18/05/2017	No	Y	Y	Y	Y	Y	Y	N	Y	Y
Maamobi	54	F	18/05/2017	No	Y	N	N	Y	Y	Y	N	Y	Y
Adenta	72	F	20/05/2017	No	N	N	N	Y	Y	Y	N	Y	Y
Adenta	21	F	17/05/2017	2011	N	Y	N	Y	Y	Y	Y	N	N
Amasaman	44	F	27/05/2017	No	Y	Y	Y	N	Y	Y		N	N
Asofa	47	F	22/05/2017	No	Y	Y	Y	Y	Y	Y	N	N	Y
Amasaman	26	F	26/05/2017	2011	Y	N	N	Y	Y	Y	N	Y	N
Pokuase	26	F	22/05/2017	2011	Y	N	N	Y	Y	Y	N	Y	N
Nima	34	M	27/05/2017	No	Y	Y	Y	Y	Y	Y	N	Y	Y
Adenta	35	F	27/05/2017	No	N	N	N	Y	Y	Y	N	Y	Y
Mamprobi	49	M	26/05/2017	2011	N	Y	N	Y	Y	Y	N	Y	Y
Nima	42	F	24/05/2017	No	N	N	N	Y	N	N	N	Y	N
Adenta	28	F	26/05/2017	2011	Y	N	N	Y	Y	Y	N	Y	N

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Ofankor	40	M	26/05/2017	2011	Y	Y	N	Y	Y	Y	N	Y	Y
Akwetey man	35	M	15/09/2017	2011	N	Y	N	Y	Y	Y	N	Y	Y

Appendix 3: Zika virus ELISA Results (Raw results)

Plate 1: Zika IgG 1 to 80

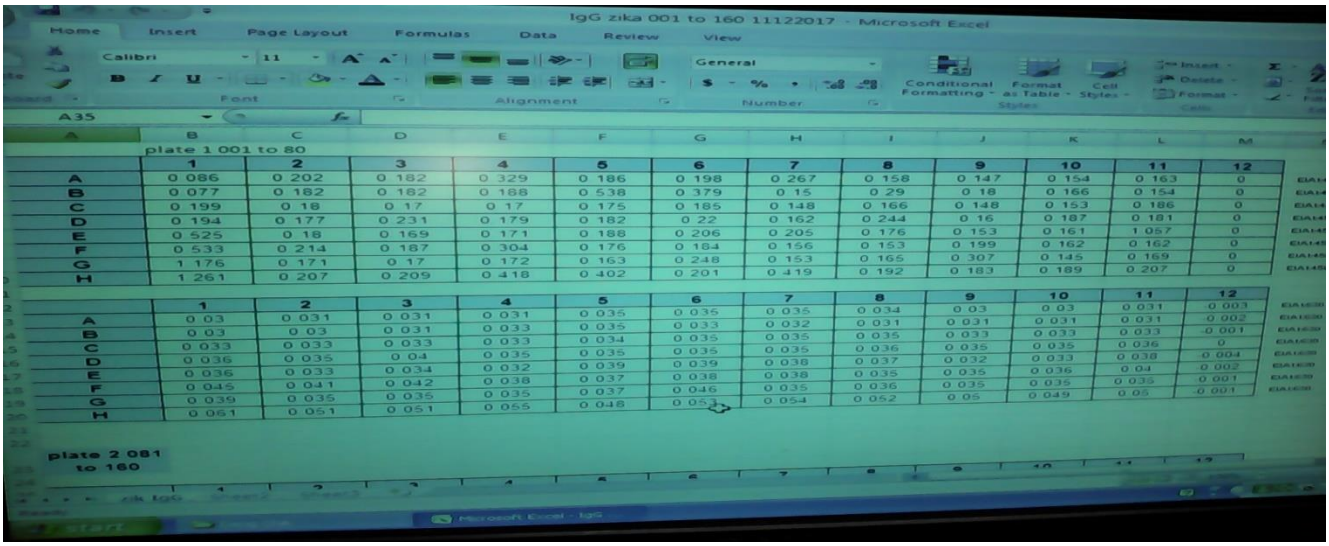

Plate 2: Zika IgG 81 to 160

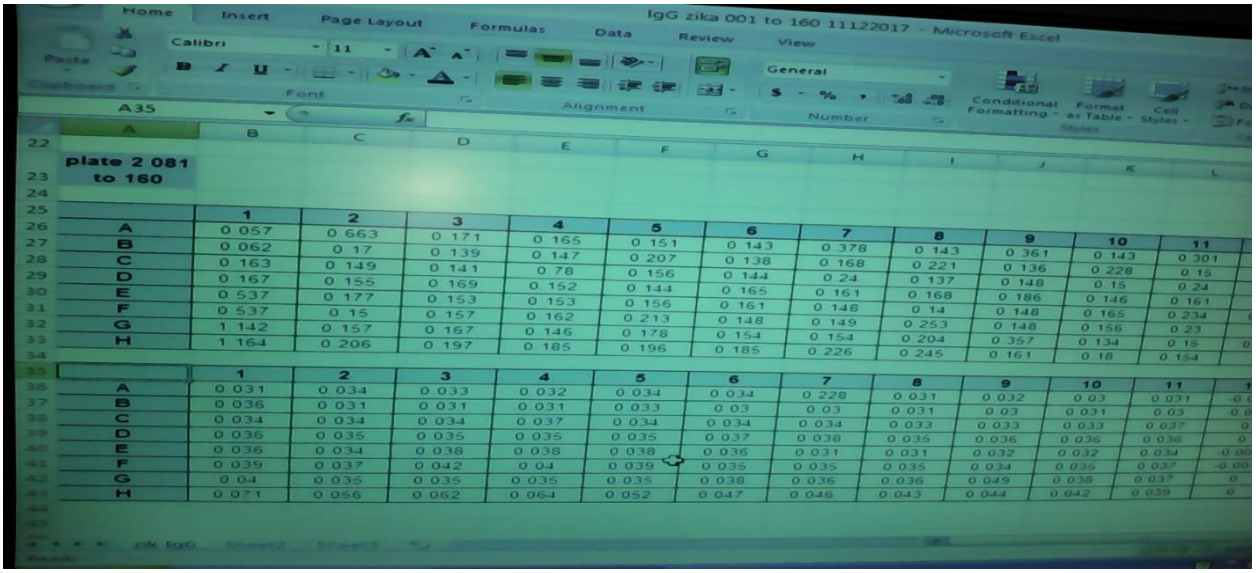

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Plate 1: Zika IgM 1 to 88

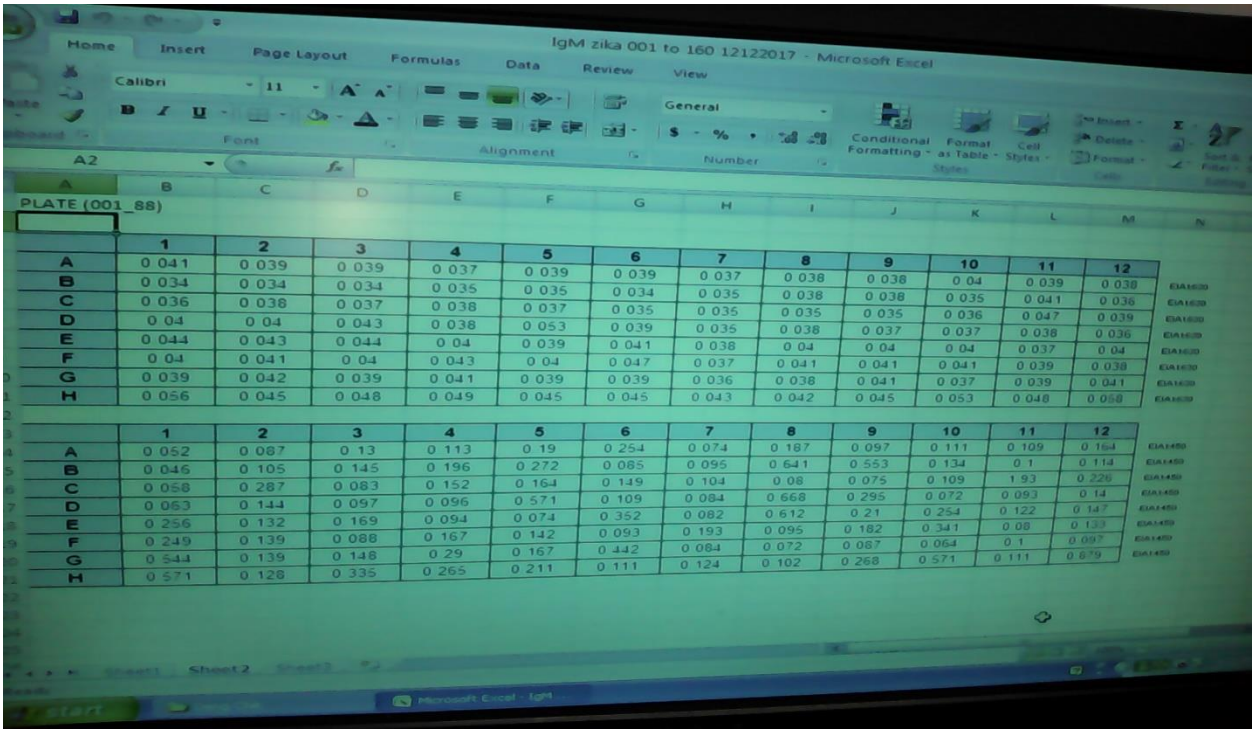

PLATE (001_88)

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 041	0 039	0 039	0 037	0 039	0 039	0 037	0 038	0 038	0 04	0 039	0 038
B	0 034	0 034	0 034	0 035	0 035	0 034	0 035	0 038	0 038	0 035	0 041	0 036
C	0 036	0 038	0 037	0 038	0 037	0 035	0 035	0 035	0 035	0 036	0 047	0 039
D	0 04	0 04	0 043	0 038	0 053	0 039	0 035	0 038	0 037	0 037	0 038	0 036
E	0 044	0 043	0 044	0 04	0 039	0 041	0 038	0 04	0 04	0 04	0 037	0 04
F	0 04	0 041	0 04	0 043	0 04	0 047	0 037	0 041	0 041	0 041	0 039	0 038
G	0 039	0 042	0 039	0 041	0 039	0 039	0 036	0 038	0 041	0 037	0 039	0 041
H	0 056	0 045	0 048	0 049	0 045	0 045	0 043	0 042	0 045	0 053	0 048	0 058

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 052	0 087	0 13	0 113	0 19	0 254	0 074	0 187	0 097	0 111	0 109	0 164
B	0 046	0 105	0 145	0 196	0 272	0 085	0 095	0 641	0 553	0 134	0 1	0 114
C	0 058	0 287	0 083	0 152	0 164	0 149	0 104	0 08	0 075	0 109	1 93	0 226
D	0 063	0 144	0 097	0 096	0 571	0 109	0 084	0 668	0 295	0 072	0 093	0 14
E	0 256	0 132	0 169	0 094	0 074	0 352	0 082	0 612	0 21	0 254	0 122	0 147
F	0 249	0 139	0 088	0 167	0 142	0 093	0 193	0 095	0 182	0 341	0 08	0 133
G	0 544	0 139	0 148	0 29	0 167	0 442	0 084	0 072	0 087	0 064	0 1	0 097
H	0 571	0 128	0 335	0 265	0 211	0 111	0 124	0 102	0 268	0 571	0 111	0 679

Plate 2: IgM 89 to 160

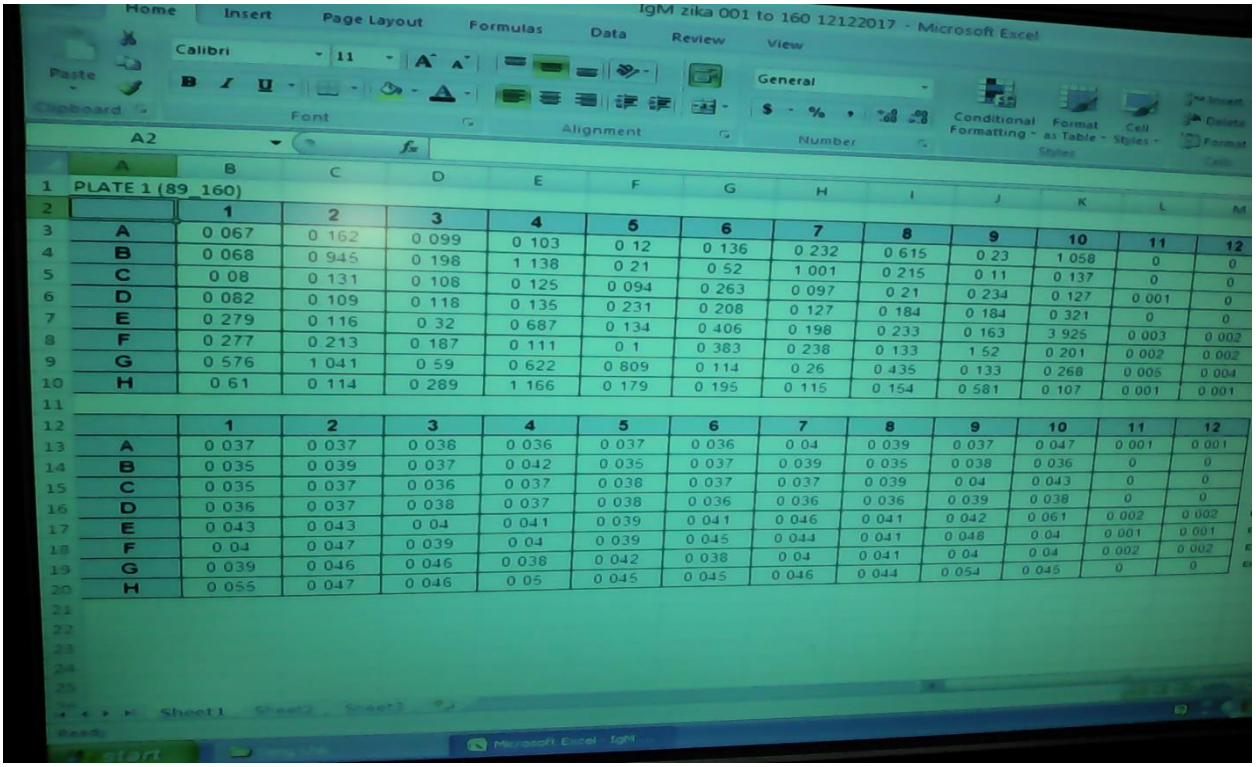

PLATE 1 (89_160)

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 067	0 162	0 099	0 103	0 12	0 136	0 232	0 615	0 23	1 058	0	0
B	0 068	0 945	0 198	1 138	0 21	0 52	1 001	0 215	0 11	0 137	0	0
C	0 08	0 131	0 108	0 125	0 094	0 263	0 097	0 21	0 234	0 127	0 001	0
D	0 082	0 109	0 118	0 135	0 231	0 208	0 127	0 184	0 184	0 321	0	0
E	0 279	0 116	0 32	0 687	0 134	0 406	0 198	0 233	0 163	3 925	0 003	0 002
F	0 277	0 213	0 187	0 111	0 1	0 383	0 238	0 133	1 52	0 201	0 002	0 002
G	0 576	1 041	0 59	0 622	0 809	0 114	0 26	0 435	0 133	0 268	0 005	0 004
H	0 61	0 114	0 289	1 166	0 179	0 195	0 115	0 154	0 581	0 107	0 001	0 001

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 037	0 037	0 038	0 036	0 037	0 036	0 04	0 039	0 037	0 047	0 001	0 001
B	0 035	0 039	0 037	0 042	0 035	0 037	0 039	0 035	0 038	0 036	0	0
C	0 035	0 037	0 036	0 037	0 038	0 037	0 037	0 039	0 04	0 043	0	0
D	0 036	0 037	0 038	0 037	0 038	0 036	0 036	0 036	0 039	0 038	0	0
E	0 043	0 043	0 04	0 041	0 039	0 041	0 046	0 041	0 042	0 061	0 002	0 002
F	0 04	0 047	0 039	0 04	0 039	0 045	0 044	0 041	0 048	0 04	0 001	0 001
G	0 039	0 046	0 046	0 038	0 042	0 038	0 04	0 041	0 04	0 04	0 002	0 002
H	0 055	0 047	0 046	0 05	0 045	0 045	0 046	0 044	0 054	0 045	0	0

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Appendix 4: Calculated Zika Virus Elisa Results

Plate 1: IgG 1 to 80

Blank	0.056	3.500512	3.091095	6.100307	3.091095	3.336745	4.749232	2.538383	2.395087	2.538383	2.702149
Negative	0.162	3.111566	3.091095	3.172979	10.29683	7.082907	2.415558	5.301945	3.050154	2.763562	2.517912
Cut-off	0.4885	3.009212	2.804504	2.804504	2.886387	3.070624	2.313204	2.681679	2.354145	2.456499	3.132037
Positive	1.1685	2.906858	3.909928	2.947799	3.009212	3.787103	2.599795	4.257932	2.558854	3.111566	2.96827
		3.009212	2.763562	2.845445	3.050154	3.418628	3.418628	2.845445	2.47697	2.620266	**20.85977**
		3.541453	2.96827	5.445241	2.845445	2.988741	2.415558	2.415558	3.357216	2.579324	2.497441
		2.784033	2.763562	2.804504	2.579324	4.135107	2.415558	2.640737	5.568066	2.251791	2.743091
		3.193449	3.234391	7.430911	7.246673	3.029683	3.009212	2.865916	2.72262	2.865916	3.21392

Plate 2: IgG 81 to 160

Blank	0.026	**12.59259**	2.762763	2.642643	2.342342	2.182182	3.003003	2.242242	6.586587	2.262262	5.405405
Neg	0.13	2.782783	2.162162	2.322322	3.483483	2.162162	2.762763	3.803804	2.122122	3.943944	2.402402
Cut	0.4995	2.302302	2.142142	**14.87487**	2.442442	2.202202	4.124124	2.082082	2.302302	-3.6036	4.064064
Post	1.09	2.402	2.682	2.342	2.182	2.562	2.462	2.662	3.003	2.202	2.502

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	75	402	683	342	182	563	462	663	003	202	503
		2.862863	2.302302	2.302302	2.362362	2.502503	2.342342	2.182182	2.322322	2.662663	4.004004
		2.262262	2.302302	2.442442	3.483483	2.262262	2.282282	4.364364	2.282282	2.422422	3.863864
		2.442442	2.642643	2.222222	2.822823	2.322322	2.362362	3.363363	6.166166	1.921922	2.262262
		3.003003	2.702703	2.422422	2.882883	2.762763	3.603604	4.044044	2.342342	2.762763	2.302302

Plate 3: IgM 1 to 88

Blank	0.0115	2.280285	4.32304	3.610451	7.173397	10.21378	1.75772	7.078385	2.80285	3.372922	3.325416	5.985748
Neg	0.0225	3.372922	5.273159	7.648456	11.25891	2.422803	2.850356	**28.64608**	**24.46556**	4.703088	2.80285	3.705463
Cut	0.2105	11.82898	2.185273	5.415677	6.033254	5.415677	3.27791	2.137767	1.900238	3.467933	**89.45368**	8.88361
Pos	0.51	4.940618	2.565321	2.755344	**24.60808**	3.325416	2.327791	**29.92874**	**12.25653**	1.662708	2.612827	4.940618
		4.228029	5.938242	2.565321	1.662708	**14.77435**	2.090261	**27.1734**	8.07601	10.16627	4.038005	5.083135
		4.655582	2.280285	5.890736	4.845606	2.185273	7.410926	2.565321	6.698337	**14.25178**	1.947743	4.513064
		4.608076	5.178147	11.82898	6.08076	**19.14489**	2.280285	1.615202	2.185273	1.28266	2.897862	2.660333
		3.942993	**13.6342**	10.26128	7.885986	3.135392	3.847981	2.850356	10.59382	**24.60808**	2.992874	**39.00238**

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Plate 4: IgM 89 to 160

Blank	0.0315	5.285412	2.663848	2.832981	3.509514	4.22833	8.118393	**24.35518**	8.160677	**42.74841**
Neg	0.0455	**38.30867**	6.807611	**46.34249**	7.399577	**20.42283**	**40.67653**	7.610994	3.044397	4.270613
Cut	0.2365	3.97463	3.044397	3.72093	2.367865	9.556025	2.536998	7.230444	8.20296	3.551797
Pos	0.546	3.044397	-11.0782	4.143763	8.160677	7.272727	3.84778	6.257928	6.131078	11.96617
		3.086681	11.83932	**27.31501**	4.016913	**15.4334**	6.427061	8.118393	5.116279	**163.3827**
		7.019027	6.257928	3.002114	2.579281	**14.29175**	8.118393	3.890063	**62.24101**	6.807611
		42.07188	**23.00211**	**24.69345**	**32.43129**	3.213531	9.302326	**16.65962**	3.932347	9.640592
		2.832981	10.27484	4.904863	5.665962	6.342495	2.917548	4.651163	**22.2833**	2.621564