SEROPREVALENCE OF ZIKA VIRUS IN SELECTED REGIONS IN KENYA

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(H56/81949/2015)

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DECLARATION OF ORIGINALITY

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SEROPREVALENCE OF ZIKA VIRUS IN SELECTED REGIONS IN KENYA

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DEDICATION

I dedicate this work to Almighty God who placed in my hands people whose support and encouragement cannot be underestimated. My darling mother, Judith Nasimiyu Kisuya, my lovely departed father, Simon Wanyonyi Kisuya, my beloved uncle, Fredrick Mukhwana Gaitano, I cherish your family kindness and support; dear Brethren Peter Meadows and Richard Harris of Dawn Christadelphian Committee (UK-Based Charity Organization), your generous contribution towards my academic journey was a source of smile I treasure so much; dear Brother Justus Mabuka and the entire Christadelphian Bible Mission (CBM), western Kenya fraternity, your ceaseless support kept me going during tough times. May Almighty God bless all the mentioned and others for their generosity towards my master's study.

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LIST OF ABBREVIATIONS AND ACRONYMS

ABTS-2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid

CDC-USA Centres for Disease Control and Prevention

CO_2-Carbon dioxide

DLSP-Diagnostics and Laboratory Systems Program

ELISA- Enzyme Linked Immunoabsorbent Assay

FBS-Foetal Bovine Serum

GBS-Guillain-Barre Syndrome

IgG -Immunoglobulin G

IgM- Immunoglobulin M

KEMRI/CDC-Kenya Medical Research Institute/Centres for Disease Control and Prevention

MNT-Microneutralization Test

Ml –millitre

Nm-nanometre

NGS-Normal Goat Serum

$NOCO_2$-Nitric oxide Carbon dioxide

OD-Optical Density

PBS-Phosphate Buffered Saline

PRNT-Plague Reduction Neutralization Test

RT-PCR-Reverse Transcription Polymerase Chain Reaction

SWOP-Sex Workers Outreach Programme

UNITID-University of Nairobi Institute of Tropical and Infectious Diseases

WNV-West Nile virus

WHO- World Health Organization

ZIKV-Zika virus

ABSTRACT

Background: The 2015-2016 Zika virus pandemic in South America stirred worldwide concern by the public and scientific community. This was due to studies linking Zika virus infection to neurological complications such as microcephaly in newborns. Zika virus is principally spread by *Aedes* mosquito species amongst jungle primates and humans. Evidence for circulation of Zika virus in East Africa necessitates surveillance studies in Kenya to benchmark efforts for monitoring, prevention, and its control.

Broad Objective: To establish seroprevalence of Zika virus in selected regions in Kenya using archived serum samples.

Study Method: A laboratory-based cross-sectional descriptive study was conducted on 582 adult human sera initially obtained from Nairobi, Eldoret, and Kisumu from 2009 to 2014, and preserved at the University of Nairobi Institute of Tropical and Infectious Diseases laboratories. The study samples were screened for anti-Zika virus antibodies by an IgG-based Enzyme Linked Immunoabsorbent Assay (ELISA). Any sample tested positive by ELISA was confirmed for the presence of specific antibodies to Zika virus by Plaque Reduction Neutralization Test (PRNT). Chi-square or Fisher's exact test was used to analyse any statistical association between proportional variation of the prevalence of anti-Zika virus antibodies and the study locations (Nairobi, Eldoret, and Kisumu), where sera were originally collected. This was facilitated by STATA for Windows version 11.2 (StataCorp, Texas, USA).

Results: Out of 135 sera from Eldoret, 135 sera from Kisumu, and 312 sera from Nairobi screened by ELISA, 5 returned positive results, 2 were from Kisumu and 3 from Nairobi. One of the two positive sera by ELISA from Kisumu tested positive for anti-Zika virus antibodies as confirmed by Zika virus PRNT, while 3 positive sera by ELISA from Nairobi, returned positive results for dengue virus as confirmed by Dengue virus PRNT. There was no statistical association between the prevalence of anti-Zika virus antibodies and the study locations (Fisher's exact test P value=0.232).

Conclusion: There was evidence of low previous exposure to Zika virus in the study population. Of the three regions in Kenya where sera for this study were obtained, only Kisumu County had one case of previous exposure to Zika virus. Epidemiological changes which might have taken place since 2013 when the sample was first collected necessitates further surveillance studies to update the country regarding the seroprevalence of Zika virus.

CHAPTER ONE

1.0 BACKGROUND INFORMATION

Zika virus (ZIKV) was first isolated from a rhesus monkey in Zika forest, near Entebbe in Uganda in 1947. For the last six decades, human cases of Zika virus infection have been reported in several countries in Sub-Saharan Africa and South East Asia. The above-mentioned cases of human Zika virus infection resulted from the spillover of ZIKV by *Aedes* mosquito species to human settlements from equatorial rain forests, where it is thought to circulate freely amongst primates (Weaver *et al.*, 2016; Wikan and Smith, 2016).

French Polynesia and Brazilian studies associating Zika virus infection to Guillain-Barre Syndrome in adults generated a lot of anxiety globally. Likewise, recent Brazilian reports linking microcephaly of infants and newborns of some infected mothers to Zika virus attracted international public health concern (Gatherer and Kohl, 2017).

Zika virus is spread primarily by *Aedes* mosquito species (Benelli, 2016). Similarly, viral RNA and/or viral proteins of Zika virus has been isolated from human blood (Gake *et al.*, 2017), semen (Musso, Roche, Robin, *et al.*, 2015), urine, vaginal fluids and breast milk of infected mothers (Chen and Tang, 2016), implying possible dissemination of ZIKV by the above named human fluids.

The global prevalence of Zika virus is often underestimated due to over 80% asymptomatic cases that remain unreported (Aggarwal *et al.*, 2016), hence skewed information with regard to its worldwide distribution and abundance. During an outbreak of Zika virus, only 20% report self-limiting febrile illness, which may be easily misdiagnosed as other common febrile diseases such as dengue fever or chikungunya (Moulin *et al.*, 2016). Human cases of febrile illness due to Zika virus infection are clinically managed through supportive care, which involves rest, fluid replacement, analgesics and antipyretics (Chen and Tang, 2016). There is ongoing pursuit of therapeutic drug and vaccine development for treatment and/or prevention of Zika virus infection by investigators. Moreover, development of better and reliable diagnostics are under investigation to boost global surveillance of Zika virus (Pierson and Graham, 2016; Shan *et al.*, 2016).

Vector control measures play an essential role in curtailing the spread of Zika virus. This requires entomological surveillance to map out areas with the high abundance of infected *Aedes* mosquito species. For instance, the presence of infected *Aedes* mosquito vectors should alarm public healthcare authorities in the concerned area for the possible outbreak of Zika virus (Benelli, 2016).

There has been circulation of Zika virus in East Africa evidenced by entomological surveillance reports (Geser, Henderson and Christensen, 1970; Royal and Tropical, 1982; Babaniyi *et al.*, 2015; Velásquez-serra, 2016; Song *et al.*, 2017), which are supported by Ugandan, Ethiopian, and Sudanian serological studies (Crabtree *et al.*, 2018; Mengesha Tsegaye *et al.*, 2018; Soghaier *et al.*, 2018). Although there has been no evidence of Zika virus in Kenya, the movement of people in Uganda, Ethiopia, Sudan, and Kenya is unrestricted. This free movement may facilitate the entry of Zika virus in Kenya. In the event Zika virus is spread in Kenya, the presence of *Aedes* mosquitoes can easily promote its spread in the country. Moreover, transovarial transmission by *Aedes* mosquitoes may result in sustained Zika virus infection and recurrent outbreaks confinement within Kenyan borders (Li *et al.*, 2017).

It is, therefore, necessary to carry out surveillance studies in Kenya to ascertain the circulation of Zika virus within its populace. To this end, the current study purposed to determine the seroprevalence of Zika virus in selected regions in Kenya by use of stored serum samples.

The study aimed at availing information regarding the possible previous circulation of Zika virus in the studied Kenyan population. Furthermore, the current study is expected to stimulate further research to generate more data about Zika virus and inform control and prevention measures to mitigate any possible outbreak of Zika virus in Kenya (Wiwanitkit, 2016b).

1.1 STATEMENT OF THE PROBLEM

Tropical climate (a geographical zone in a range of latitudes between 23.5^{o} south and 23.5^{o} north, with relatively high temperatures throughout the year) predominating in some parts of Kenya support growth and multiplication of *Aedes* mosquito species (Liang, Gao and Gould, 2015). Coastal and Lake Victoria regions in Kenya receive relatively high amounts of rainfall, thereby allowing luxuriant growth of diverse vegetation, which offer breeding and hiding places for different mosquitoes. Furthermore, the presence of littered waste containers such as storage jars and used-up tyres which can hold some dirty water in urban centres such as Eldoret, Kisumu, and Nairobi in Kenya due to poor drainage and sewerage systems offer breeding grounds for *Aedes* mosquitoes (Weaver and Reisen, 2010). Consequently, *Aedes aegypti*, which are container breeders and live in close proximity to humans, can easily aid transmission of Zika virus in such kind of environment, in the event of its introduction through global travel and trade.

There has been frequent occurrence of arboviral infections in Kenya such as dengue fever and chikungunya (Sutherland *et al.*, 2011; Lutomiah *et al.*, 2016). Viral infection due Zika virus, chikungunya virus, and dengue virus share similar clinical manifestation such as self-limiting febrile illness, which may confound human cases of Zika virus infection (Shapshak *et al.*, 2016).

The growing population in urban centres in Kenya is another important factor with regard to the spread of Zika virus. If it happens Zika virus is introduced in such places, ZIKV can easily spread from one person to another through infected female *Aedes* mosquitoes, or from infected person to susceptible people through blood transfusion exercises among other human activities in such overcrowded areas (Rodriguez-Morales, Bandeira and Franco-Paredes, 2016).

The true seroprevalence of Zika virus in Kenya is unknown and diagnosis of febrile illness is a challenge to date. Some of the unidentified febrile illness in some of the healthcare facilities may be due to Zika virus which is not routinely suspected. Serological and entomological surveillance studies in some of the potential places are crucial in yielding scientific results to benchmark sound responses to some of these enquiries.

1.2 JUSTIFICATION

The study intended to determine the level of previous human exposure to Zika virus in the above mentioned urban centres among people generally considered healthy. This is because, 80% of people infected with Zika virus do not show any clinical symptoms (Aggarwal *et al.*, 2016). Moreover, clinical manifestations such as self-limiting febrile illness ensuing from human infection by chikungunya virus, dengue virus, and ZIKV closely resemble each other. There has been circulation of dengue and chikungunya viruses in Kenya (Paul *et al.*, 2016) ,without any published studies on Zika virus. While the Dengue virus and Chikungunya virus studies availed information regarding their prevalence, possible previous human exposure to Zika virus has not yet be done or done and not yet published.

Diagnosis and differentiation of Zika virus, dengue virus, and chikungunya virus in most developing countries such as Kenya is a challenge, hence, there is scanty information regarding disease burden posed by dengue virus, chikungunya virus, besides, the newly emerging Zika virus in Kenya (Waggoner and Pinsky, 2016).

The association of microcephaly in the newborns and Guillain-Barre Syndrome in adults with Zika virus infection in the 2015-2016 ZIKV pandemic in South America generated health care concern worldwide.

Besides other investigations, this association necessitated surveillance studies to aid in determining the possible healthcare risk posed by the presence of Zika virus (Song *et al.*, 2017).

The findings by this study shall be published in a peer-reviewed journal, thereby availing information to the general public. This is expected to stir further research to generate more information with reference to Zika virus .This shall inform control and prevention measures such as initiation of vector control strategies in affected areas to mitigate any possible outbreak of Zika virus in Kenya.

1.3 RESEARCH QUESTIONS

1. Were Kenyans in the selected regions (Eldoret, Kisumu and Nairobi) previously exposed to Zika virus?
2. Is there any statistical association between prevalence of Zika virus and the study locations?

1.4 BROAD OBJECTIVE

To establish seroprevalence of Zika virus in selected regions in Kenya using archived adult human sera sourced from the University of Nairobi Institute of Tropical and Infectious Diseases laboratories.

1.5 SPECIFIC OBJECTIVES

1. To determine seroprevalence of Zika virus in selected regions in Kenya.
2. To test any statistical relationship between the study locations and seroprevalence of Zika virus.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 NOMENCLATURE AND CLASSIFICATION OF ZIKA VIRUS

Zika virus (ZIKV) is a re-emerging mosquito-borne flavivirus belonging to the family of viruses called *Flaviridae* (Mackenzie, Gubler and Petersen, 2004). Yellow fever virus, West Nile Virus, Japanese encephalitis virus, Tick-borne encephalitis virus and dengue virus are among other species of global health importance in the genus *Flavivirus*, besides ZIKV. Bite of infected arthropods such as mosquitoes and ticks aid transmission of flaviviruses (Yun and Lee, 2017). Some mosquito-borne flaviviruses are categorized based on their clinical symptoms. Encephalitis flaviviruses (Japanese encephalitis virus and West Nile Virus) are associated with neurological disorders, whereas non-encephalitis flaviviruses (Yellow fever virus and dengue virus) are linked to haemorrhagic fevers. Transmission of non-encephalitis flaviviruses is depended on presence and abundance of *Aedes* mosquito species as well as jungle primates such as apes and baboons. Conversely, spread of encephalitis flaviviruses is facilitated by *Culex* mosquito species and vertebrate hosts such as birds (Petersen and Marfin, 2005) as illustrated in figure 1.

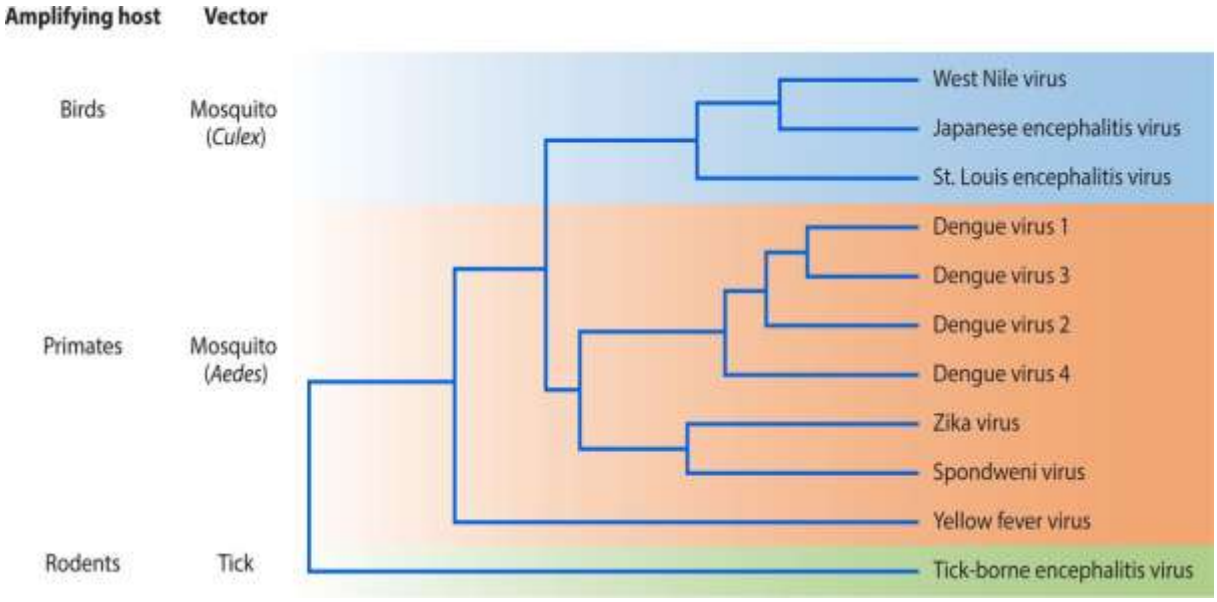

Figure 1:Schematic representation of Flaviviruses (Lazear and Diamond, 2016)**.**

Scientific data accumulated over years from genomic comparisons and phylogenetic studies suggest divergence of Zika virus into two main strains: Asian Zika virus strain and African Zika virus strain (Vorou, 2016). The Zika virus strain which has been circulating in Latin America (Malone *et al.*, 2016), closely resembles the Asian Zika virus strain isolated in French Polynesia during 2013-2014 outbreak (Faye *et al.*, 2014).

2.2 STRUCTURE AND CHARACTERISTICS OF ZIKA VIRUS

2.2.1 The Structure of Zika virus

Zika virus is made up of non-segmented linear positive sense single stranded RNA genome enclosed in a nucleocapsid. Lipid bilayer acquired from infected host cell incorporated in Zika virus capsid protein outlines an icosahedral enveloped capsid shell encircling the nucleocapsid. There are 180 copies for each of E and M proteins, constituting outer coat of Zika virus (Kostyuchenko *et al.*, 2016).The above described components among others are well illustrated in figure 2 below.

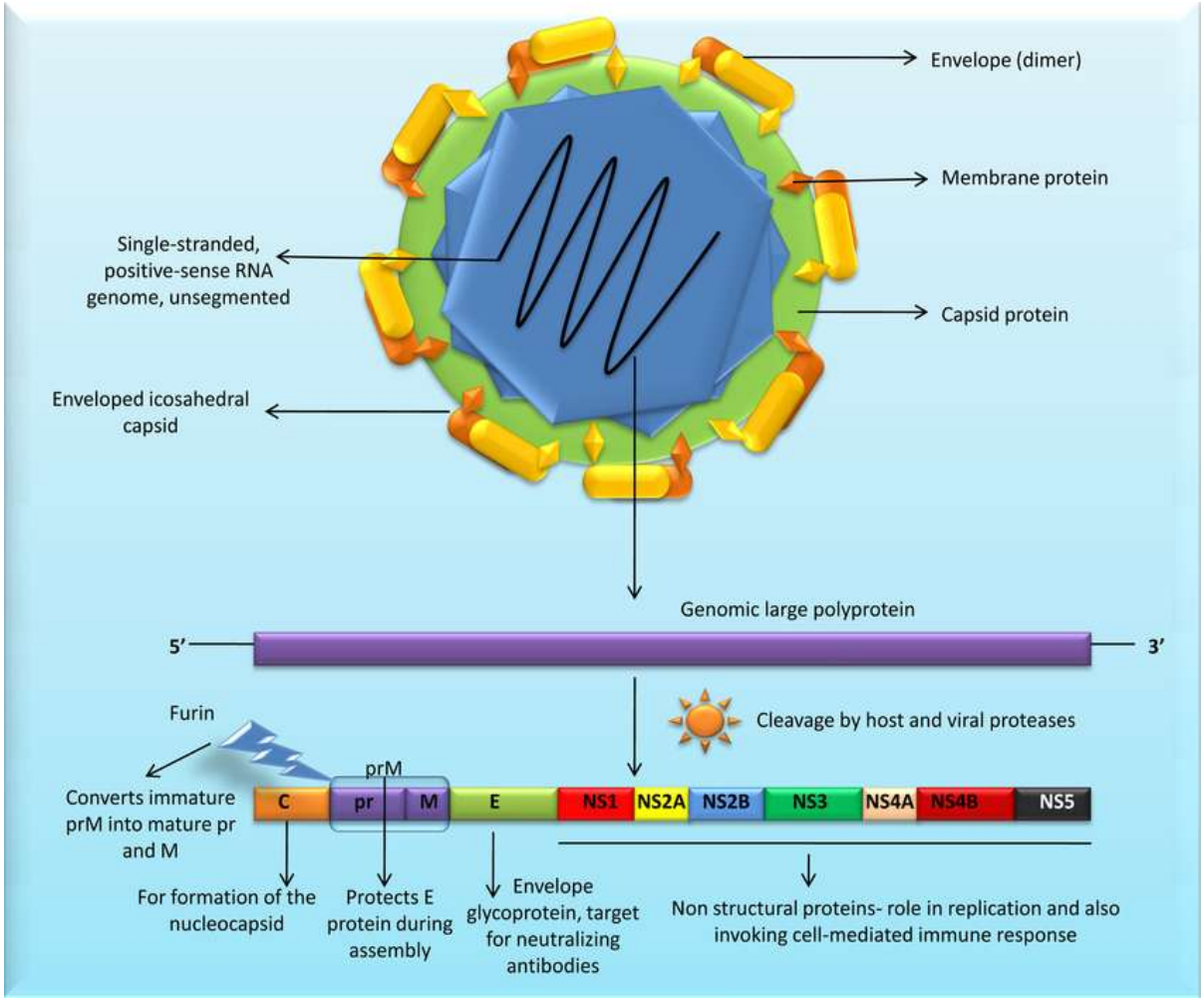

Figure 2: Zika virus structure (Singh *et al.*, 2016)**.**

2.2.2 Life cycle of Zika virus

Zika virus, like other flaviviruses, attach to susceptible and permissive host cell surfaces by help of E protein, followed by internalisation of Zika virus particle by endosome (Davis *et al.*, 2006). Low pH in the host cell endosome induces fusion of E protein with endosomal membrane. Subsequently, the above fusion process interrupts Zika virus nucleocapsid, thereby discharging Zika virus RNA genome into host cell cytoplasm (Gillespie *et al.*, 2010).

Host cell translation apparatus facilitate translation of Zika virus RNA genome into a single polyprotein. Protease enzymes from the host cell and Zika virus cleave the above polyprotein into structural and non-structural viral proteins as shown in figure 2 above. Some non-structural proteins from the above translation process, catalyse synthesis of negative-sense single stranded RNA from the positive copy, and thereafter replication of more positive sense Zika virus RNA genomes (Zhang *et al.*, 2003).

Several constituents of Zika virus particle generated by the above translation and replication processes assemble inside endoplasmic reticulum of the host cell cystosol. Capsid protein integrated with lipid bilayer acquired from host cell encircles assemblage of components of Zika virus particle, as they move via Golgi apparatus networks, where post-translation modification takes place. Exocytosis of infected host cell expels mature Zika virus particles, ready to attack other susceptible and permissive host cells (Mackenzie and Westaway, 2001). Figure 3 below summarizes description of life cycle of Zika virus inside infected cells.

More and new similarities and differences in replication and translation of Zika virus and other flaviviruses await future investigations (Stadler *et al.*, 1997).

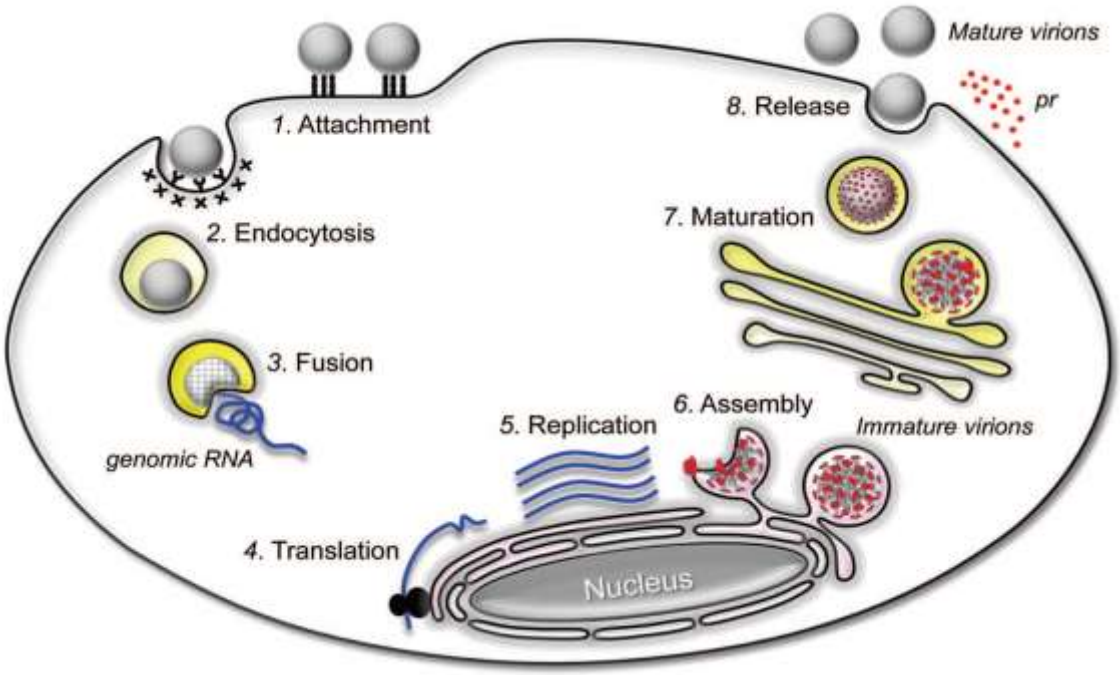

Figure 3:Diagrammatic life cycle of Zika virus (Yun *et al.*, 2014)**.**

2.3 HISTORY AND EPIDEMIOLOGY OF ZIKA VIRUS

For the last six decades, Zika virus has been circulating within some countries in Asia and Africa, since its discovery in Uganda in 1947. In 2007, Zika virus emerged in Yap Islands, before spreading to French Polynesia and other Pacific Islands in 2013-2014. Zika virus was reported in Latin America in 2015. The first case of Zika virus infection was reported and confirmed by CDC in the United States of America by 2016. The worldwide historical circulation and outbreaks of Zika virus over years is summarized in figure 4 below.

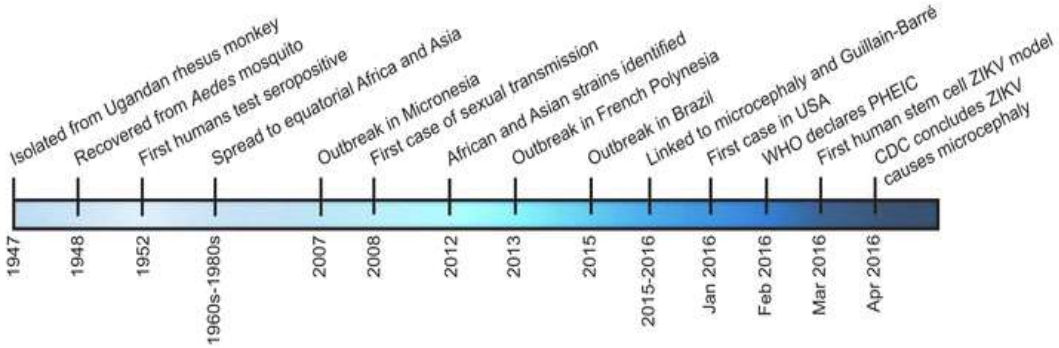

Figure 4: Historical spread of Zika virus over years (Qian *et al.*, 2017)**.**

2.3.1 Isolation and seroprevalence of Zika virus preceding 2007

During a Yellow fever Study, funded by Rockefeller Foundation, in April 1947, there was an unusual viral isolate (MR-766) from a rhesus monkey, in Zika forest, near Entebbe in Uganda (Zhou *et al.*, 2016). Later on, the same virus was isolated from *Aedes africanus* mosquitoes captured in January 1948 from the same forest (Al-qahtani *et al.*, 2007). Subsequently, the viral isolate (MR-766) was named "Zika virus" after the place, where it was first discovered and described (Wikan and Smith, 2016).

In 1952, results of a serological survey conducted in Uganda, reported cases of human Zika virus infection. Prevalence of antibodies against Zika virus in the study carried out on serum samples of Kampala resident patients presented with febrile illness was approximately 10% to 20%. Two years later, there was a jaundice outbreak in eastern Nigeria, where Zika virus was isolated from three patients (Al-qahtani *et al.*, 2007).

Studies conducted for almost fifty years (Posen *et al.*, 2016) apparently point to possible geographical restriction of Zika virus to a narrow equatorial belt running across some African and Asian countries (Gyawali, Bradbury and Taylor-robinson, 2016).

2.3.2 Outbreak of Zika virus in 2007 in Micronesia

In April 2007, an outbreak of Zika virus outside Africa and South East Asia was reported and confirmed in the Islands of Yap in the Federated States of Micronesia, where serum samples from patients presenting with keratoconjunctivitis, arthralgia and rash, initially thought to be

due to chikungunya, dengue fever, or Ross River disease, had Zika virus. There were neither deaths nor hospitalization of the infected people during the above mentioned Zika virus outbreak (Kool *et al.*, 2009).

2.3.3 Outbreak of Zika virus in 2013-2014 in French Polynesia and Pacific Islands

There was an outbreak of Zika virus in French Polynesia commencing October 2013. According to healthcare reports, 11% of French Polynesia citizenry was infected with Zika virus, with some of them presenting with Guillain-Barre Syndrome (Saiz *et al.*, 2016). From French Polynesia, ZIKV spread to New Caledonia, and the Cook Islands. Human cases of Zika virus were also reported in Australia in 2012, which were mainly contributed by travellers from Indonesia and other countries where Zika virus was in active circulation. Similarly, New Zealand experienced increased human cases of Zika virus infection as a result of infected travellers from the affected countries (Wikan and Smith, 2016).

2.3.4. Outbreak of Zika virus in 2015-2016 in Latin America

Several countries in Latin America experienced an onslaught of Zika virus pandemic between 2015 and 2016 (Weaver *et al.*, 2016). In April 2015, the first case of ZIKV in Brazil was reported and confirmed by Brazilian healthcare authorities. Thereafter, Zika virus was reported by the Brazilian neighbouring countries in South America, North America and the Caribbean Islands. Strong association between Zika virus infection and microcephaly (Brasil *et al.*, 2016) was first reported in Brazil by February 2016 (Faria *et al.*, 2016). Increased cases of human Zika virus infection in Brazil (Hennessey, Fischer and Staples, 2016), associated with microcephaly as well as Guillain-Barre Syndrome generated a lot of healthcare concern worldwide (Gyawali, Bradbury and Taylor-robinson, 2016). Some countries issued travel warnings to their citizens as a result of ongoing active Zika virus circulation in Brazil (Petersen *et al.*, 2016). This was meant to caution and discourage a lot of athletes and tourists expected to travel to the 2016 Summer Olympic Games hosted in Rio de Janeiro, Brazil (Bogoch *et al.*, 2016).

During the Zika virus pandemic in South America, an enormous number of human cases of Zika virus infection were reported and confirmed by healthcare authorities in the affected countries. Several theories have been advanced to explain this rare phenomenon. Genetic mutations of the recent Latin American Zika virus strain augmented its infectivity of *Aedes aegypti* vectors. Subsequently, more infectious vectors infecting a large population compared to the old African Zika virus strain (Liu *et al.*, 2017). Moreover, mutated Latin American Zika virus strain was found to be more severe and caused more cases of microcephaly in laboratory neonatal mice than closely related old Asian Zika virus strain (Zhang *et al.*, 2017).

This may explain why more cases of human Zika virus infection as well as cases of microcephaly were recorded in this particular outbreak. In addition, cross-reaction of non-neutralizing antibodies between dengue virus and Zika virus might have played a role in disease severity witnessed in the recent ZIKV pandemic in the Latin American populace (Paul *et al.*, 2016). Some investigators postulate that antibody-dependent Zika virus enhancement (ADE) mechanism increased severity of Zika virus infections in Latin American scenario, where non-neutralizing cross-reactive antibodies generated during dengue virus infection, might bind to specific Zika virus surface epitopes (Dejnirattisai *et al.*, 2016). This enhances uptake of Zika virus by Fc-receptor bearing cells, and subsequently increased the number of infected cells contributing to high viral burden and stimulation of a more vigorous host immune response encompassing inflammatory cytokines and mediators, some of which lead to the development of fever and chills clinically observed among some Zika virus patients (Keasey *et al.*, 2017). The above described antibody-dependent Zika virus enhancement (ADE) mechanism was supported by naivety of Latin American population to Zika virus (Brasil *et al.*, 2016) and existing scientific reports backing previous predominance of dengue virus in South America (Malone *et al.*, 2016).

Figure 5 below captures worldwide geographical and historical description of Zika virus in this section.

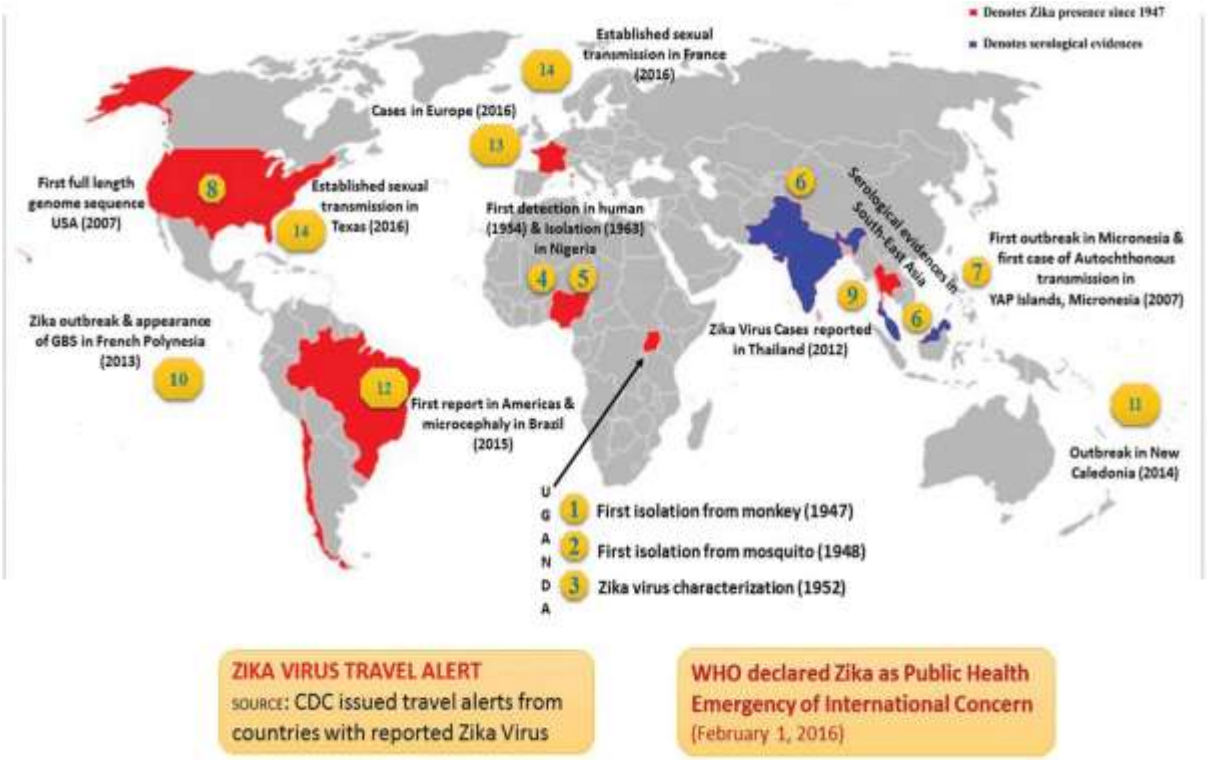

Figure 5: Global distribution of Zika virus (Singh *et al.*, 2016)**.**

2.4 SPREAD OF ZIKA VIRUS

Mosquitoes infected with Zika virus can potentially transmit it to vulnerable people. Besides, infected mothers can transmit ZIKV to their foetuses through placenta or to their infants at delivery or during breastfeeding. Zika virus can also spread through sexual intercourse as well as during blood transfusion exercises. Developing control strategies against Zika virus is quite challenging due to multiple routes of transmission as demonstrated in figure 6 below.

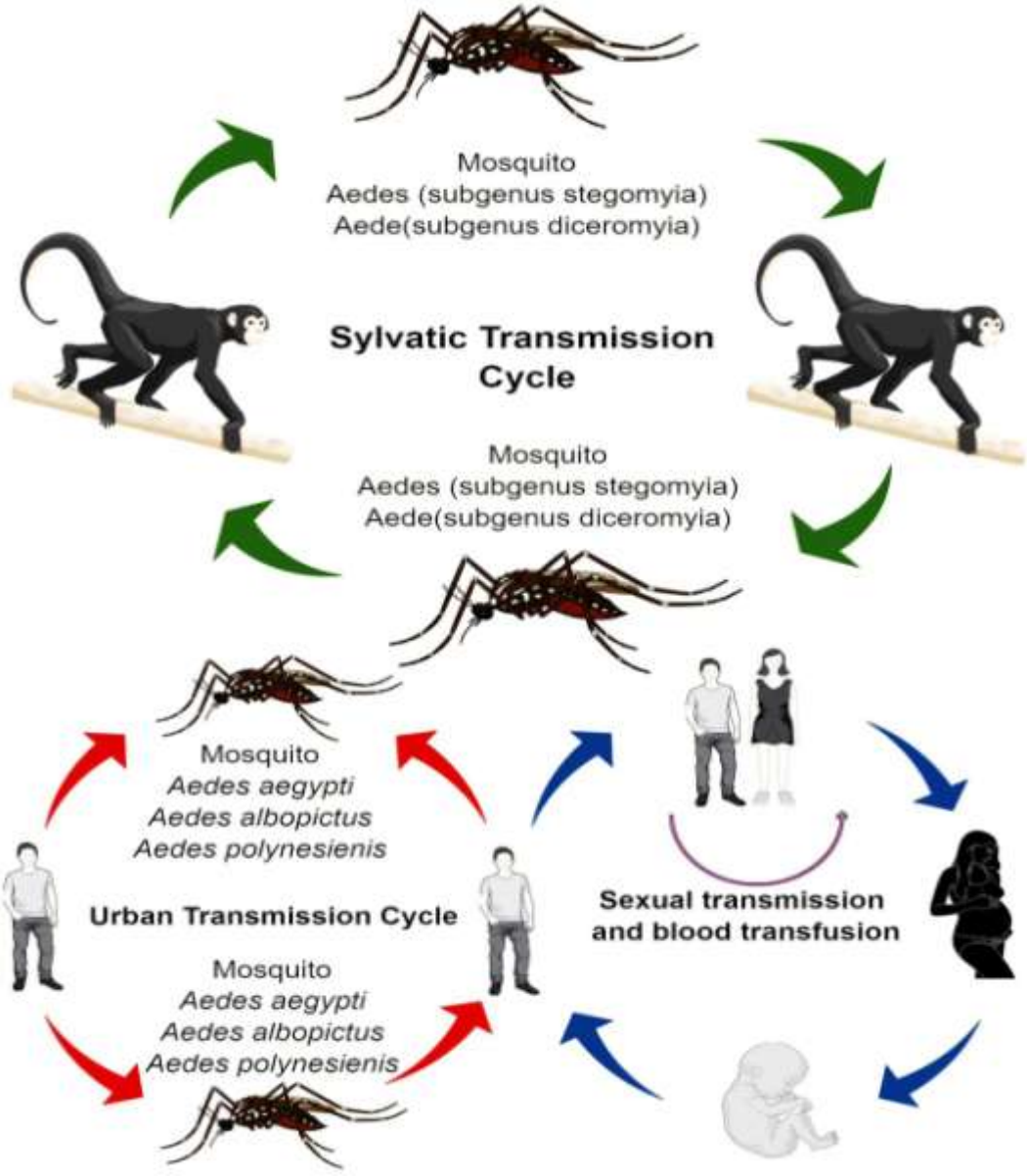

Figure 6: Routes of Zika virus transmission (Rather *et al.*, 2017)**.**

2.4.1 Spread of Zika virus via mosquitoes

There are two separate phases in which infected *Aedes* mosquitoes aid spread of Zika virus. Forest-dwelling mosquitoes play an important role in maintenance of Zika virus amongst jungle primates such as monkeys, baboons, and apes in a sylvatic phase. In an urban cycle, Zika virus is spread between susceptible people and urban mosquitoes in human settlements. In some rare occasions, arboreous mosquitoes propagate Zika virus to susceptible people due to their nearness to forests (Weaver *et al.*, 2016).

Several *Aedes* mosquito species within the tropics harbour Zika virus. For example, *Aedes africanus* mosquitoes associated with the sylvatic cycle, facilitate transmission of Zika virus among jungle primates in some Asian and African equatorial rain forests(Weaver *et al.*, 2016).

Figure 7: *Aedes* mosquitoes (Gathany, 2018)**.**

In the urban cycle, Zika virus is predominantly spread by *Aedes aegypti* besides *Aedes albopiticus* mosquitoes shown in figure 7 above (Velásquez-serra, 2016). The two *Aedes* species are generally active at daytime hours, and are commonly found in the tropics and subtropics, with *A. albopiticus* extending their habitats further into cool temperate regions (Mombo *et al.*, 2014). The geographical expansion of *Aedes albopictus* mosquitoes have been facilitated by international trade in used tyres. During transportation of used tyres from Asian countries, eggs of *Aedes albopictus* mosquitoes get deposited in tyres especially when they contain rainwater (Vorou, 2016). *Aedes aegypti* mosquitoes preferentially stay adjacent to human settlements where they facilitate spread of Zika virus in the event of an outbreak. The immature stages of *Aedes aegypti* mosquitoes are commonly housed in water-filled sites such as empty used containers near human homesteads. Studies hypothesis that most female *Aedes aegypti* mosquitoes usually stay around people's houses where they hatch their young ones, which later on develop into adults. Thus, human beings play an essential role in spreading ZIKV within and between communities (Liang, Gao and Gould, 2015). Additionally, infected

female *Aedes aegypti* mosquitoes can transmit Zika virus to their offspring thereby maintaining ZIKV in an endemic area in what is called transovarial transmission. Transovarial transmission is responsible for persistence of Zika virus in endemic regions during inter-epidemic periods (Li *et al.*, 2017).

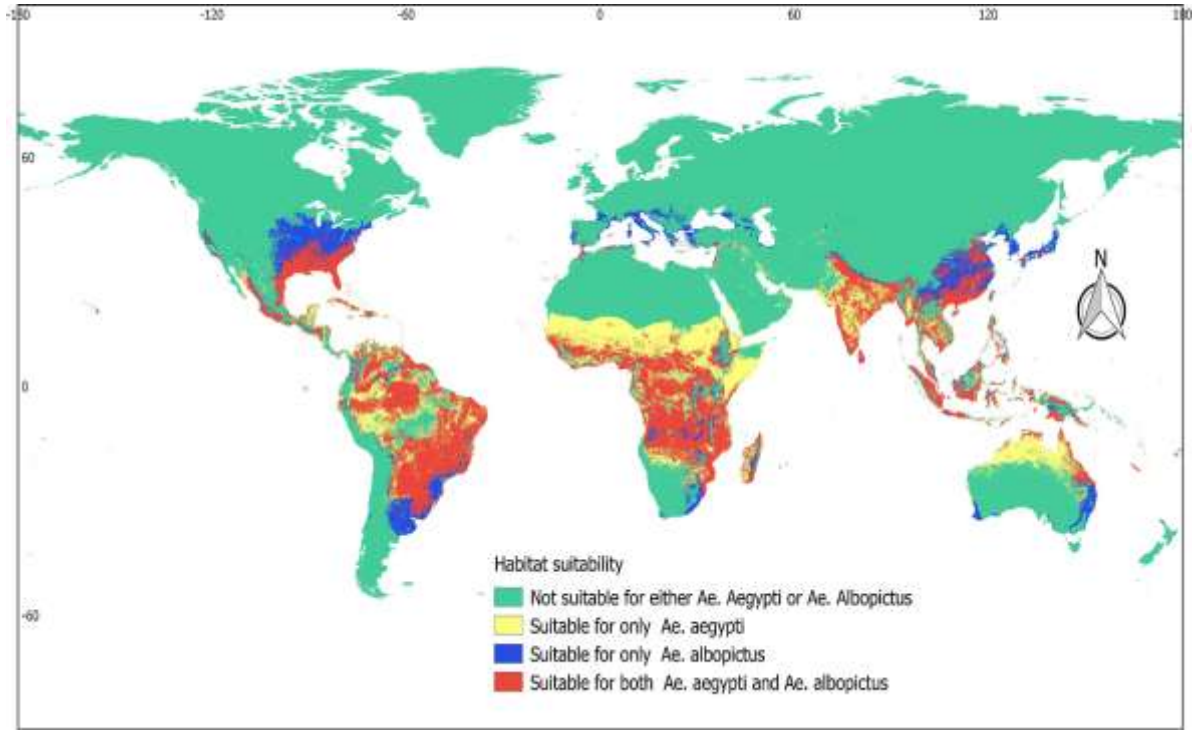

Figure 8: Global distribution of *Aedes* mosquitoes (Leta *et al.*, 2018)**.**

The subsequent figure 8 above shows global suitability in addition to probably distribution and abundance of *Aedes aegypti* and *Aedes albopictus* mosequitoes within the tropics and sub-tropics, and nicely summarizes description of the same within this sub-section. Moreover, *Aedes hensilli* and *Aedes polynensis* aided spread of Zika virus during outbreaks in Yap islands and French Polynesia respectively (Ledermann *et al.*, 2014).

In addition, some *Culex* mosequito species are experimentally capable of Zika virus infection in the laboratory, implying that they may be candidates for the spread of Zika virus. However, the role of *Culex spp* in propagation of Zika virus is yet to be determined in field experiments (Guo *et al.*, 2016).

2.4.2 Spread of Zika virus via alternate routes

Diverse modes of transmission of Zika virus between and within human populace have been documented. Vertical transmission of Zika virus from infected mother to her child through placenta before birth or during delivery as well as during breastfeeding is a case in point. Likewise, blood transfusion with infected blood donor and sexual intercourse with infected partner are alternative ways, hypothesized to aid spread of Zika virus.

Some infected mothers having foetus with brain abnormalities had traces of Zika virus in their urine, blood and amniotic fluid. Traces of Zika virus RNA and/or proteins were found in the brain, serum and placenta of newborns or aborted foetus of the above mothers. This strongly supports a possible transmission of Zika virus from infected mothers to their foetuses or newborns (Driggers *et al.*, 2017; Wen, Song and Ming, 2017; Zhang *et al.*, 2017). Similarly, breast milk of some infected mothers was found with traces of Zika virus (Besnard *et al.*, 2014), implying a potential route for propagation. Moreover, reports of possible sexual transmission of Zika virus has been evidenced by presence of Zika virus RNA and/or proteins in seminal and vaginal fluids (Miner and Diamond, 2017). Additionally, other human fluids such as throated and urinal discharges of infected people may aid dissemination of Zika virus (Musso, Roche, Nhan, *et al.*, 2015; Malone *et al.*, 2016; Rodriguez-Morales, Bandeira and Franco-Paredes, 2016; Wikan and Smith, 2016). Blood transfusion exercises can potentially spread Zika virus from infected blood donors to susceptible recipients (Musso *et al.*, 2014).

Identification and characterization of risk factors involved in the above alternate routes for the spread of Zika virus await further studies.

2.5 DISEASE MANIFESTATION

There is a wide diversity of clinical symptoms manifested by 20% of people infected with Zika virus. There is growing scientific evidence that strongly associates Zika infection with microcephaly in infants born to infected mothers and Guillain-Barre syndrome in adults.

2.5.1 Common signs and symptoms

About 20 - 25% of people infected with Zika virus develop mild, self-limiting febrile illness, with an incubation period of 4 - 10 days (Al-qahtani *et al.*, 2007; Chen and Tang, 2016; Singh *et al.*, 2016). Common clinical symptoms exhibited by some patients infected with Zika virus include slight pyrexia, popular rash, oedema, joint and muscle pain. Moreover, haematospermia, hearing difficulties, thrombocytopenia, and subcutaneous bleeding have also been clinically observed in some cases of human Zika virus infection (Shapshak *et al.*, 2016). These symptoms normally show up shortly during viremic phase, with long persistence of arthralgia that may last as long as four weeks or more (Foy *et al.*, 2011).

2.5.2 Guillain-Barre syndrome in adults

This is a neurological defect orchestrated by stimulation of auto-antibodies mounting an immune response against components of one's own peripheral nervous system. This is characterized by soreness, muscular dystrophy, palsy or mortality in some rare occasions (Goodfellow and Willison, 1916).

The association between Zika virus and Guillian-Barre Syndrome (GBS) was strongly suspected during an outbreak of Zika virus in French Polynesia (Musso *et al.*, 2014) and Brazil (Musso and Gubler, 2016). Human cases of GBS increased tremendously during the outbreak of Zika virus in French Polynesia. Similarly, a Brazilian study observed 41 (98%) of 42 Guillain-Barre Syndrome patients had antibodies (IgM/IgG) to Zika virus, in comparison to 54 (55%) of 98 controls who did not have any other febrile illness. So far, Guillian-Barre Syndrome due to Zika virus has been momentary, and many patients eventually recover. There is intense research in progress to unravel mechanism through which Zika virus infection contributes to the development of Guillian-Barre Syndrome (Oehler *et al.*, 2014).

2.5.3 Microcephaly in newborns

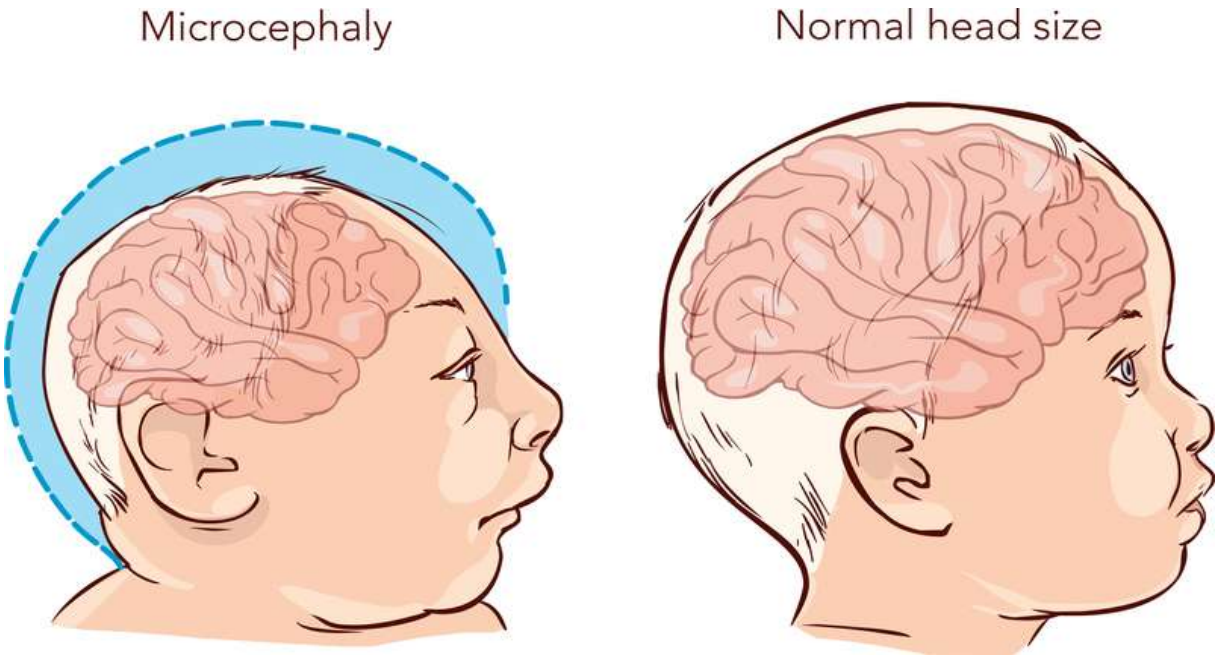

Figure 9: Microcephaly compared to infant normal head size(Watson, 2018)**.**

Microcephaly is a neurological birth disorder occasioned by improper development of central nervous system during pregnancy which may lead to newborns with abnormally small heads as illustrated above in figure 9 (Klase, Khakhina and Schneider, 2016). This may be as a result of diminishing nerve cells, synapse linkages and nerve fibres during neural tissue development (Faria *et al.*, 2016). Brain injuries, congenital infections, genetic mutations and chemical agents of the foetus during pregnancy are known factors contributing to the development of microcephaly (Zhu *et al.*, 2016).

The causal association between microcephaly and Zika virus infection surfaced in Brazil. This was due to very large number of cases of newborns with microcephaly reported between September 2015 and July 2016. During this period alone, there were 8,301 cases of microcephaly (Rasmussen *et al.*, 2016).

Moreover, retrospective French Polynesian study (Jouannic *et al.*, 2016) and prospective Brazilian study connected causal association between Zika virus infection and microcephaly in infants born to infected mothers (Brasil *et al.*, 2016). Additionally, traces of Zika RNA and/or proteins were found in the amniotic fluid, diverse cells of microcephalus foetuses and infants of infected mothers. Furthermore, isolation of specific IgM antibodies to Zika virus was observed by some investigators within microcephalous neonatal blood and neural tissues (Besnard *et al.*, 2014).

Upcoming epidemiological studies will unveil more and maybe new neurological and non-neurological complications associated with Zika virus infection (Benelli, 2016; Brasil *et al.*, 2016; Musso *et al.*, 2016; Weaver *et al.*, 2016; Song *et al.*, 2017).

2.6 HUMAN IMMUNE RESPONSES TO ZIKA VIRUS

There are ongoing scientific studies to underpin how humans mount innate and adaptive immune response against Zika virus. Meanwhile, the available well studied flavivirus models enrich us on how the two arms of the immune system may coordinate to facilitate clearance of Zika virus in immunocompetent individuals. Nevertheless, complications may arise in immunocompromised persons and in some rare occasions, Zika virus can overwhelm the immune system and cause disease even in healthy individuals.

2.6.1 Innate Immune Response

Bite from infected female *Aedes spp* mosquito inoculates Zika virus into human skin. Following inoculation, Zika virus replicates in local dendritic cells beneath the skin (Hamel *et al.*, 2015), from where it is taken to local lymph nodes by dendritic cells. While in the local lymph nodes, Zika virus particles interact with resistant macrophages as well as naïve lymphocytes (Xie, Shan and Shi, 2016). The resident macrophages can phagocytose Zika virus particles as well as generate interferons that can halt their spread (Brinton, 2014). From local lymph nodes, Zika virus particles which are neither neutralized nor destroyed by lymphocytes spread to the spleen, where they replicate and multiply abundantly to high titres. While antibodies and macrophages in the spleen neutralize, destroy and may even eradicate Zika virus particles, some of them escape and enter bloodstream contributing to secondary viremia (Pierson and Graham, 2016). From the bloodstream, some unbound Zika virus particles can spread and infect neurons in some rare occasions leading to neurological disorders (Jain, Coloma and García-sastre, 2016). Furthermore, complement system, during early phase of infection may be activated by lectin pathway or alternative pathway to augment neutralization, opsonization and lyses of infected cells,

which may eventually result in the clearance of Zika virus from the system of infected host (Morrison and Diamond, 2017). However, Zika virus particles present in the bloodstream of infected mother may spread across the placenta causing damages to the foetus, which may lead to development of microcephaly (Mlakar *et al.*, 2016).

2.6.2 Adaptive Immune Response

The neutralizing immunoglobulin (IgM) antibodies appear early in the adaptive immune response to Zika virus, followed later on by immunoglobulin (IgG) antibodies. In the initial phase of Zika virus infection, IgM antibodies appear in abundance in the bloodstream. However, within few weeks of infection, IgM antibodies titres dwindle, making it difficult to be used as disease markers. The IgG antibodies appear between first and second week of infection, and persist in the bloodstream for years. The effecter T helper cells aid priming and class switching of specific antibodies through production of cytokines such as interleukins (IL-4) (Keasey *et al.*, 2017). The effecter T helper cells are also involved in recruiting macrophages and other immune cells to boost the clearance of Zika virus from the infected person via secretion and release of Type II interferons and interleukins (IL-2) (Enfissi *et al.*, 2016). Moreover, presence of either IgM or IgG antibodies trigger classical pathway of the complement system which may also boost the clearance of Zika virus through opsonization, neutralization, and lyses of infected cells (Asif *et al.*, 2017; Conde *et al.*, 2017).

More scientific investigations in future shall establish differences and similarities of human immune response to Zika virus and other flaviviruses. Furthermore, such research efforts will also improve and better the diagnostic tools available for Zika virus surveillance and investigation.

2.7 EVASION OF IMMUNE SYSTEM BY ZIKA VIRUS

Zika virus, like other pathogens, avoids detection or manipulates immune surveillance system to facilitate its growth and multiplication, which may result in disease manifestation. For instance, ZIKV NS5 protein antagonizes Type I interferon pathway, meant to resist its establishment in host cells. The above mentioned Zika virus NS5 protein interrupts interferon stimulating genes (ISG) thereby halting the generation of molecules to stall replication of Zika virus within the infected cells (Manuscript and Nanobiomaterials, 2013). Consequently, Zika virus evades innate immune system response mediated via Type I interferon pathway and establishes infection in various susceptible and permissive cells of the infected host (Asif *et al.*, 2017). This postulation is supported by laboratory experiment done using mice models. High viral load was observed in the neural tissues and testicles of experimental mice deficient of Type I interferon system and was severely affected by Zika virus infection in comparison

to mice with intact Type I interferon system (Lazear *et al.*, 2016). This is consistent with studies linking Zika virus infection with neurological diseases as well as possible transmission of Zika virus through sexual intercourse with an infected partner (Atkinson *et al.*, 2016; Rodriguez-Morales, Bandeira and Franco-Paredes, 2016; Morrison and Diamond, 2017; Song *et al.*, 2017). Moreover, Zika virus infection via beta interferon pathway upregulate major histocompatibility complex I molecules, thereby obstructing killing of infected host cells by natural killer cells (Histocompatibility, 2017).

Similarly, the immune response mounted against Zika virus may be counteracted by Zika virus NS1 protein. Presence and accumulation of ZIKV NS1 protein in infected host cell cystosol induces inflammation, which provokes the further release of inflammatory cytokines by monocytes and macrophages. Subsequently, accumulation of inflammatory cytokines may contribute to vascular leakage of the infected endothelial cell plasma (Asif *et al.*, 2017; Conde *et al.*, 2017). This may explain why tissue and organ damage observed in microcephalous foetus and infants of infected mothers (Cugola *et al.*, 2016; Miner and Diamond, 2017).

Moreover, monoclonal antibodies generated against some portions of dengue virus E protein (EDI/II) bind similar epitopes of incoming ZIKV EDI/II but fail to neutralize it. The cross-reaction process described above enhances the uptake of Zika virus by Fc-receptor bearing cells in what is called antibody-dependent enhancement (ADE), which boosts replication of Zika virus and activation of cross-reactive memory T cells. Consequently, generation and release of non-specific cytokines contribute to the severity of Zika fever as clinically observed in some ZIKV patients (Karin Stettler, Martina Beltramello, Diego A. Espinosa, Victoria Graham, Cassotta, Siro Bianchi, Fabrizia Vanzetta, Andrea Minola, Stefano Jaconi, Federico Mele, Mathilde Foglierini, Mattia Pedotti, Luca Simonelli, Stuart Dowall and Elena Percivalle, Cameron P. Simmons, Luca Varani, Johannes Blum, Fausto Baldanti, Elisabetta Cameroni, Roger Hewson, Eva Harris, Antonio Lanzavecchia, Federica Sallusto, 2016; Asif *et al.*, 2017). Likewise, monoclonal antibodies generated against ZIKV E protein (EDII) stimulate cross-reactive memory T cells which induce similar phenomenon already described above (Karin Stettler, Martina Beltramello, Diego A. Espinosa, Victoria Graham, Cassotta, Siro Bianchi, Fabrizia Vanzetta, Andrea Minola, Stefano Jaconi, Federico Mele, Mathilde Foglierini, Mattia Pedotti, Luca Simonelli, Stuart Dowall and Elena Percivalle, Cameron P. Simmons, Luca Varani, Johannes Blum, Fausto Baldanti, Elisabetta Cameroni, Roger Hewson, Eva Harris, Antonio Lanzavecchia, Federica Sallusto, 2016).

New and more investigations shall unveil further ways used by ZIKV to elude the human immune system. Such research pursuits will also better our understanding and facilitate the strategic development of better diagnostic tools, therapeutic drugs, and vaccines against Zika virus.

2.8 DIAGNOSTIC TOOLS FOR ZIKA VIRUS

Over the decades, Zika virus had been neglected, until its recent association with microcephaly and Guillian-Barre Syndrome (Aggarwal *et al.*, 2016). This has necessitated development of diagnostic tools, some of which are quite expensive. Surveillance studies of Zika virus have been intensified in some countries to avail timely information with regard to prevalence and transmission dynamics. WHO urges countries to always share any information regarding Zika virus. This is aimed at facilitating control efforts against spread of Zika virus globally (Gake *et al.*, 2017). Unfortunately, many countries may not have sufficient personnel and resources to implement the above desired directive. This challenges efforts geared towards control and prevention of Zika virus menace (Faye *et al.*, 2014). Moreover, Zika fever may be clinically confounded with other febrile vector-borne parasitic and viral diseases like malaria, dengue fever, and chikungunya. Surveillance efforts to Zika virus infection globally are generally poor and a lot of information regarding its abundance and epidemiological distribution is not known (Petersen *et al.*, 2016). Furthermore, the available diagnostic tools are not affordable to many. These factors complicate and challenge diagnosis and investigation of Zika virus(Noor and Ahmed, 2018).

Laboratory confirmation of Zika virus plays an important role in its diagnosis and management. This is because, Zika fever clinically resembles other febrile infections such as dengue fever (Keasey *et al.*, 2017). There is also marked cross-reaction of non-neutralizing antibodies by closely related flaviviruses such as dengue virus and Zika virus (Pialoux *et al.*, 2007).

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) is a powerful technique that plays an important role in diagnosis and characterization of the different strains of Zika virus. This method usually amplifies specific tiny pieces of nucleic acid of Zika virus into large quantities, which can then be used to confirm its presence in an infected person. Moreover, the amplified pieces of nucleic acid from RT-PCR technique can also be compared with other known nucleic acid sequences to establish specific strains of Zika virus that may be responsible for an outbreak. This technique is useful during an acute phase of infection. This is because; Zika virus can be detected in circulating blood cells, some tissues and organs for

about seven days subsequent to infection (Faye *et al.*, 2008; Balm *et al.*, 2012; Aliota *et al.*, 2016; Enfissi *et al.*, 2016; Mlakar *et al.*, 2016; Wiwanitkit, 2016a; Crabtree *et al.*, 2018).

Apart from RT-PCR, Zika virus infection can also be determined by presence of specific antigen and/or antibody in human fluids especially blood. This constitute serological methods such as Enzyme Linked Immunoabsorbent Assay (ELISA), which are essential for diagnosis of Zika virus infection beyond acute phase of infection (Faye *et al.*, 2008). Presence of IgM, which persists for some weeks following acute phase of infection before disappearance from blood circulatory system, indicates recent infection with Zika virus. IgG, which appears later during and after acute phase of infection, and persists for years in circulation, confirms prior exposure to Zika virus (Waggoner and Pinsky, 2016).

There are difficulties in interpreting results from serological assays due to cross-reactivity of antibodies among flaviviruses. For example, positive results to Zika virus may also be due to infection by dengue virus. Plaque reduction neutralization test (PRNT) is a gold standard serological method used to confirm and differentiate Zika virus infection from other flaviviruses. However, PRNT is laborious, involves handling of live viruses, expensive, time consuming as it may take up to a week to be performed, tedious in sourcing standardized reagents that are often not available, and is not commonly carried out. Samples tested positive to Zika virus and are found negative to dengue virus by ELISA tests may be interpreted as a presumptive Zika virus infection in settings where it is not possible to execute PRINT (Musso and Gubler, 2016). There is also a challenge with the interpretation of PRINT results. For instance, primary exposure to any closely related flavivirus through natural infection or vaccination, results in a more vigorous secondary antibody response to the previous infecting flavivirus than response to the current one. Subsequently, interpretation of PRINT results vary and in some occasions may be quite doubtful (Waggoner and Pinsky, 2016).

In addition, RT-PCR and immunohistochemical tests are used to diagnose Zika virus infection in tissues of aborted foetus and full-term infants who die shortly after birth (Rasmussen *et al.*, 2016). Additionally, ultrasonography has been useful in supplementing clinical findings in the diagnosis of microcephaly (Honein *et al.*, 2017).

2.9 MANAGEMENT OF ZIKA VIRUS INFECTION

Healthcare facilities in affected areas have been offering supportive care to ZIKV patients such as rest, administration of fluid replacement, analgesics and antipyretics (Attaway *et al.*, 2017). Efforts are currently underway to develop a vaccine (Shan *et al.*, 2016) as well as therapeutic drug against Zika virus infection (Miner and Diamond, 2017).

Meanwhile, vector control is the main option to limit spread of Zika virus. This entails control measures for *Aedes* mosquitoes to minimize transmission. Management of mosquito breeding habitats like drainage of swamps, stagnant pools of water, removal of outdoor water containers, plant pots and empty cans decrease breeding grounds to *Aedes* mosquitoes. Use of insecticides and larvicides in homes and outdoor environment is useful in control of Zika virus. Avoidance of outdoor activities which expose people to mosquito vectors, personal protective measures like sleeping under treated mosquito nets, use of mosquito repellants and wearing protective clothing minimize exposure to ZIKV vectors (Wong, Poon and Wong, 2016).

In addition, avoidance of unprotected sex with a partner at risk of Zika virus infection should be encouraged. Similarly, travel limitation by expectant mothers to areas with high risk of transmission of Zika virus should be observed. Figure 10 on the next page pinpoints world areas with different levels of Zika virus transmission.

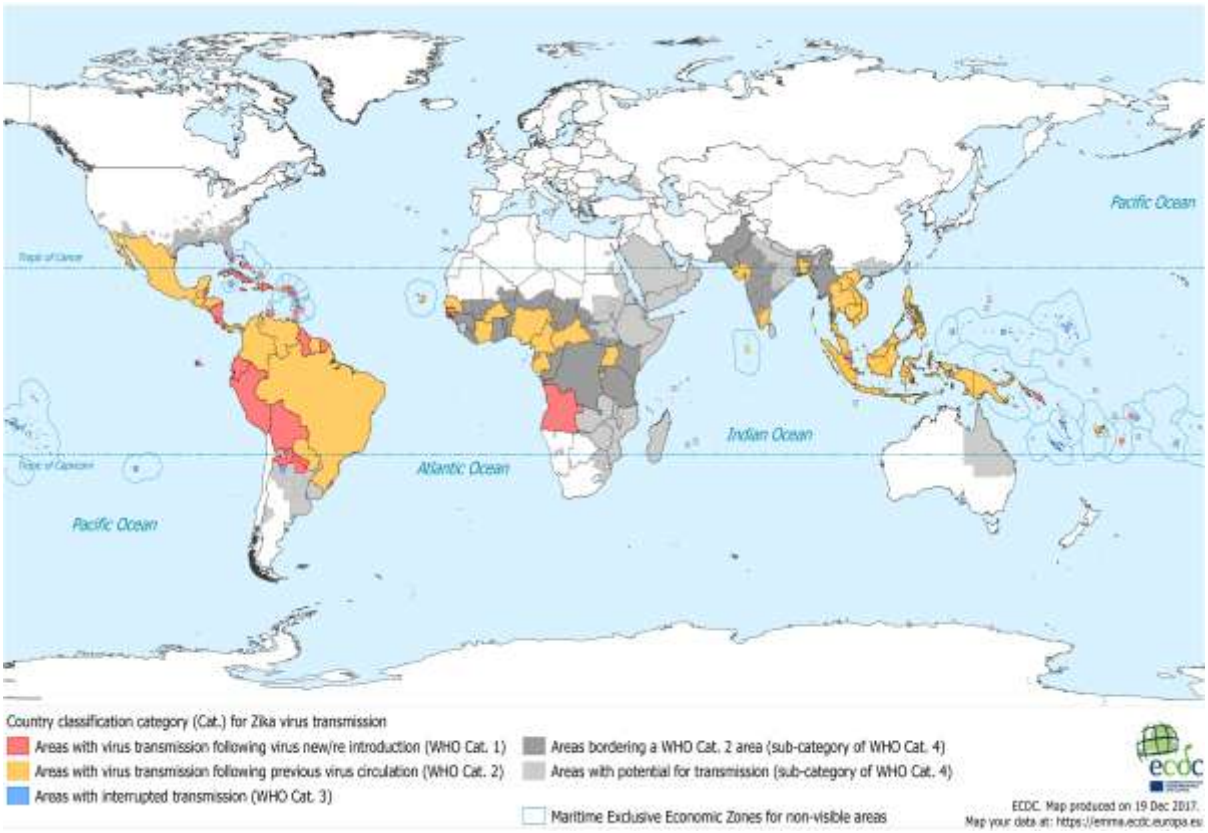

Figure 10: Levels of Zika virus transmission (Weiss Robert & Plante Beth, 2018)**.**

The control measures discussed above aim at lessening exposure to Zika virus as well as manage its spread between and within communities. Even so, each of the above mentioned approaches in curtailing spread of Zika virus has sizeable limits. For instance, although communities are mobilized in several occasions to control breeding sites to *Aedes aegypti* mosquitoes, inconsistent participation of some households and presence of extensive

inhabitants for reproduction and multiplication of *Aedes aegypti* mosquitoes in modern urban settings renders this strategy ineffective (Barrera-pe *et al.*, 2018). Moreover, control programmes to dengue fever utilize peridomestic insecticide spraying during outbreaks, but its efficacy as vector control intervention has not been well exemplified scientifically (Lenhart *et al.*, 2008). While applications of larvicides and indoor residual spraying have been efficacious in some settings, it has had little success in others (Powers *et al.*, 2016).

The above enlisted limitations call for an integrated vector control and prevention strategy, supported by timely diagnosis of Zika virus as well as sharing of the latest correct information by each country, with development of a rapid response to Zika virus which involves all within any given community (Wong *et al.*, 2013).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY DESIGN

This was a laboratory-based cross-sectional descriptive study. Healthy adult human sera archived at the University of Nairobi Institute of Tropical and Infectious Diseases (UNITID) laboratories were used to establish previous exposure of studied Kenyans to Zika virus.

3.2 STUDY AREAS

The samples were originally derived from adult human subjects from Kisumu, Eldoret and Nairobi in Kenya. Nairobi is a metropolitan city attracting Kenyans from all corners of the country as well as foreigners from East Africa and beyond. Eldoret and Kisumu are located in the Rift valley and Nyanza regions of Kenya respectively. There has been circulation of dengue virus in Nairobi, Rift Valley, Nyanza and Coastal regions of Kenya (Ochieng *et al.*, 2015). Moreover, entomological studies conducted in Kenya confirmed presence of *Aedes* mosquitoes which facilitate transmission of dengue virus besides Zika virus in Nyanza (Kisumu), Rift Valley (West Pokot) and Nairobi (Agha *et al.*, 2017; Arum *et al.*, 2018).

3.3 STUDY POPULATION

The study utilized archived sera initially collected from blood donors from Regional Blood Transfusion Centres (RBTC) in Kisumu, Eldoret, and Nairobi. The RBTC samples were earlier on used in an unpublished study to establish dengue virus hotspots in the mentioned Kenyan urban centres. Moreover, the study used serum samples originally obtained from the Sex Workers Outreach Program (SWOP) clinics in Nairobi. The SWOP samples were part of an ongoing HIV/AID study in Nairobi, Kenya. The selected samples had been well preserved at the UNITID's deep freezers laboratories at -80^0C.

3.4 SAMPLE SIZE DETERMINATION

The minimum sample size for this study was estimated based on the Cochran formula. Due to lack of published prevalence studies on Zika virus in the country, 50% was chosen as anticipated seroprevalence of Zika virus antibodies, and was used to calculate the minimum study sample size for the current study (Israel, 2013).

$$N_O = \frac{Z^2PQ}{E^2}$$

Where;

N_o-minimum number of sample required

Z-standard normal distribution at 5% significance level (1.96)

P-anticipated prevalence of Zika Virus antibodies (50%)

E-degree of precision (5% or 0.05)

$$No=\frac{1.96^2\,(0.5)\,(1-0.5)}{0.05^2}$$

$$=384.16$$

With a 95% confidence interval and a 5% degree of precision, a minimum sample size of 385 was arrived at. However, the study utilized 582 to increase the precision for the estimated seroprevalence.

3.5 SAMPLING METHOD

Convenient sampling was used to identify and integrate 582 archived human sera from UNITID laboratories. The samples comprised those from Regional Blood Transfusion Centres (RBTC) in Kisumu (n=135; December 2013-January 2014), Eldoret (n=135; January-February 2013) and Nairobi (n=100; August-September 2013). Likewise, sera from Sex Workers Outreach Program (SWOP) clinics in Nairobi (n=250; March 2009-November 2012) were incorporated in this study. The study samples were stratified on the basis of the location where they were originally collected, year of sample collection, and whether they were derived from blood donors or SWOP clinics.

3.6 LABORATORY PROCEDURES

Five hundred and eighty-two study samples were processed for anti-Zika virus antibodies using IgG ELISA. Microneutralization test was carried on all samples tested positive and equivocal by ELISA. Furthermore, plaque reduction neutralization test was conducted on all sera tested positive by ELISA.

3.6.1 (IgG) Enzyme Linked Immunoabsorbent Assay (ELISA)

Study samples were thawed by bringing them to room temperature at least one hour before actual processing and thoroughly mixed up by vortexing. During laboratory processing, 2 microlitre (µl) of each SWOP sample was diluted in 200 µl of the sample buffer supplied by Euroimmun™ at the ratio of 1:101, resulting in 202 µl sample diluent. RBTC samples were pooled, where 5 samples were mixed together before their dilution in the sample buffer at the ratio of 1:20.2. For each pool, 2 µl of individual RBTC sample was added in a pooled well, totalling 10 µl per pool, and then diluted in 192 µl of the supplied sample buffer, resulting in 202 µl sample diluent. One hundred (100) µl of calibrator 2 was pipetted into its designated calibrator 2 well; 100 µl of positive control was also added into its positive control well; 100 µl of negative control was pipetted into its negative control well; and 100 µl of each prepared

sample diluent was added into their designated wells. All wells in use were covered with protective foil and incubated for 60 minutes at 37°C±1°C**.** After the incubation period**,** protective foil was removed and reagent wells washed by an automated washer 3 times with 450 µl per well of working strength wash buffer. The 96-microplate well was gently tapped onto a dry paper towel with its openings facing downwards to remove any residual wash buffers. Thereafter, 100 µl per well of enzyme conjugate was pipetted into the microplate wells, covered with protective foil and incubated for 30 minutes at room temperature (18°C to 25°C). Thereafter, the protective foil was removed from the microplate, reagent wells washed and dried as in the first washing session. Next, 100µl per well of Chromogen solution was dispensed into microplate wells and placed in a dark and non-working incubator for 15 minutes at room temperature (18°C to 25°C). Later on, 100µl per well of stop solution was added into microplate wells. Subsequently, Optical Density (OD) of each microplate was determined at 450 nanometre (nm) wavelength of the microplate ELISA reader within 30 minutes. The resultant OD values were captured by a camera. During ELISA processing, 6 out of 582 study samples without OD values as captured by the microplate ELISA reader at 450 nm wavelength were excluded from the analysis.

3.6.2 Interpretation of (IgG) ELISA results

Laboratory study results were evaluated by calculating a ratio of OD values of the study sample, positive control and negative control subdivided by OD values of calibrator 2. The ratio was calculated using the following formula:

$$\text{Ratio} = \frac{\text{OD value of control or study sample}}{\text{OD value of calibrator 2}}$$

Laboratory result for the studied sample was considered negative if its ratio was <0.8. A ratio of $\geq$ 0.8 to <1.1 was considered equivocal, and most of such samples were retested. The test result was considered positive if its ratio was $\geq$ 1.1.

3.6.3 Microneutralization Test (MNT)

A 96-microplate was seeded with 100 µl per well of vero cells in growth media and incubated at 37^{o}C and 10% CO_2 for 1 day to allow stabilization of a monolayer. Thirty-five (35) µl of each study sample was diluted in 665 µl of growth media at ratio of 1:20, resulting in 700 µl sample diluent. Two hundred and twenty (220) µl sera dilution per well were added into triplicate wells in row H of an empty 96-well plate with the rest filled with 110 µl per well of growth media. Serial dilution of 110 µl sera diluent per well was carried out commencing

from row H through row B to yield two-fold dilutions, with two fold sera dilutions ranging from 1:20 to 1:1280. The last sera dilution of 110 µl per well was discarded from row B. Row B and A remained empty to serve as virus control and blank, respectively. Zika virus was diluted in growth media at the dilution of 1:10,000 (dilution ratio determined to yield between 50 and 100 plaques). One hundred and ten (110) µl per well of diluted Zika virus was pipetted into sera diluent plates from row H up to row B (served as virus control). Serum and Zika virus mixture was incubated at room temperature for 2 hours. Afterwards, vero cells incubated the day before were inoculated with 200 µl per well of serum and Zika virus mixture and incubated at 37^{o}C and 10% CO_2 for 3 days. Next, supernatant of cell culture was discarded and the cells fixed with 200 µl per well of ice cold fix solution (1:1 Ethanol-Methanol) in a chemical hood and incubated for 30 minutes at -20^{o}C.Thereafter, the fixed cells were washed 5 times using phosphate buffered saline (PBS) to remove the fixative in an automated washer machine. Later, 300 µl per well of PBS with 10% Normal Goat Serum (NGS) was added in each well for blocking and incubated for 30 minutes. After that, blocking was discarded without washing. One hundred (100) µl per well of 4G2 diluted in PBS and 10% NGS at the dilution of 1:4000 was added into culture plates and incubated for 2 hours at 37^{o}C ($NOCO_2$). Later on, the culture plates were washed 5 times with PBS as described in earlier session. One hundred (100) µl per well of goat-anti-mouse-HRP diluted in PBS and 10% NGS at the dilution of 1:5000 was pipetted into culture plates and incubated for 1 hour at 37^{o}C ($NOCO_2$). Subsequently, culture plates were washed 5 times with PBS as detailed in first washing session. One hundred (100) µl per well of ABTS substrate solution was added into culture plates and incubated at room temperature for 30 to 60 minutes until the virus control wells reached an optical density (OD) of 0.8 -1.3 at 405nm wavelength while inside in a microplate reader.

3.6.4 Interpretation of MNT Results

Study results encompassing OD values were entered in a prepared computer application calculation spread sheet programme. Mean standard deviation and cut-off OD values were automatically calculated. Moreover, neutralization curves were generated utilizing the entered OD values. The study sample was considered negative when the end point titre was reported as <40.This indicated absence of neutralization of Zika virus in sera dilutions resulting in OD values greater than the cut-off value. Similarly, end point titre of >1280 for a study sample was considered positive. This implied presence of neutralization of Zika virus in sera dilutions contributing to OD values less than the cut-off value.

3.6.5 Plaque Reduction Neutralization Test (PRNT)

A 6-well microplate was seeded with 3 ml per well of vero cells in growth media and incubated at 37^{o}C and 10% CO_2 for 1-3 day to allow stabilization of a monolayer. Thirty-five (35) µl study sera was diluted in 315 µl phosphate buffered saline (PBS) and 30% foetal bovine serum (FBS), resulting in 350 µl sample diluent at the ratio of 1:10. Subsequently, two fold dilutions was done by adding 350µl serum into first titre tube and serially diluted 175 µl from it to the next one down 5 tubes each column, where each had already been filled with 175µl of PBS and 30% FBS. The last dilution of 175µl was discarded, with two fold sera dilutions ranging from 1:10 to 1:320. Then, 100 µl of reference virus was diluted into 100 µl of PBS and 30% FBS. Viral dilutions were then carried out to dilution of 1:10,000 (dilution ratio determined to yield between 50 and 100 plaques). Next, each designated tube was mixed with 175 µl of diluted reference virus and mixed well by vortexing. Back titration of reference virus was conducted by mixing equal volumes of PBS and 30% FBS and test viral dilution. Back titration of the reference viral dilution was done at the dilution of 1:100,000. (dilution ratio determined to ensure appropriate plaque count has been used). The mixture of virus, serum, and back-titration was incubated for 2 hours at room temperature. Following incubation, excess culture media was removed leaving just a small volume to avoid drying from 6-well microplates, after which, 150 µl per well of virus and serum mixture was pipetted into duplicate designated wells. Separate 6-well microplate was filled with 150 µl per well of back titration, 150 µl per well of reference virus diluted at the dilution of 1:10,000 with the last one being pipetted with 150 µl per well of PBS and 30% FBS to serve as negative control in duplicate. The inoculants of the virus serum mixture and controls were incubated at room temperature for 1 hour with frequent movement (every 10 to 15 minutes) to prevent the monolayer from drying. Meanwhile, an agar overlay medium was prepared during incubation period by mixing equal volumes of 1% agarose and 2x miller's Ye-Lah overlay and kept at 37%. Subsequent to incubation, 4 ml of agar overlay was pipetted into each well and upon its solidification about 15-30 minutes; 6-well microplates were incubated at 37^{o}C and 10% CO_2 for 3 days. Next, supernatant of cell culture was discarded and the cells fixed with 200 µl per well of ice cold fix solution (1:1 Ethanol-Methanol) in a chemical hood and incubated for 30 minutes at -20^{o}C. Following the fixation of cells, 1 ml per well of 2.2% neutral red solution in PBS was pipetted into incubated well plates. The stained plates were then returned into the incubator for 24 hours. Thereafter, plaques were counted and marked with an indelible marker with the plate held upside down on a light illumination box.

The counted plagues were averaged in duplicate wells in study plates as well as the control plate.

3.6.6 Interpretation of PRNT Results

Percentage (%) plaque neutralization was determined by the following formula:

$$\% \text{ Plaque Neutralization} = \frac{\{\text{No. Virus control well plaques} - \text{No. Study well plaques}\}}{\text{No. Virus control well plaques}} \times 100$$

Percentage plaque neutralization of study sample of $\geq$ 90% was considered positive implying presence of neutralizing antibodies against the challenge virus. Those study samples with percentage plaque reduction less than 90% were regarded negative indicative of absent neutralizing antibodies against the tested virus.

3.7 DATA MANAGEMENT AND ANALYSIS

Each study sample was given unique serial identification number. These serial numbers were used for identification and traceability of samples during laboratory processing. Hard copies of the laboratory results were stored securely in locked cupboards.

Similarly, soft copies of laboratory results were captured by Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA, USA) in password protected computer, which was later on exported to STATA for Windows version 11.2 (StataCorp, Texas, USA). Frequencies of laboratory results were run in STATA to ensure consistency and accuracy. These results were saved on a flash disk; another set of results was stored in a drop box whose password was only known by principal investigator.

Data analyses of laboratory results were performed using STATA. The main interest of this study was antibodies against Zika virus. Anti-Zika virus status was described as positive, negative or equivocal based on the results of Enzyme linked Immunoabsorbent assay (ELISA), microneutralization test (MNT) and Plaque reduction neutralization test (PRNT).

Data on proportions was compared using Chi-Square or Fisher‘s exact test. The mentioned tests were run to illustrate proportional variations of anti-Zika virus antibodies in relation to study locations. All tests were carried out at 5% level of significance.

3.8 ETHICAL CONSIDERATIONS

This study was reviewed and approved by Kenyatta National Hospital-University of Nairobi Ethics and Research Committee (KNH-UoN ERC: P307/04/2016).

CHAPTER FOUR

4.0 RESULTS

4.1 PREVALENCE OF ANTI-ZIKA (IgG) ANTIBODIES

Out of 577 study samples screened for anti-Zika virus antibodies by NS1-IgG ELISA, 5 returned positive results, 2 were from Kisumu area and 3 from Nairobi. The proportion of RBTC samples that were positive by ELISA from Kisumu {1.5% (95% CI: 0.1-5.2; 2/134)} was similar to the proportion of SWOP samples from Nairobi {1.4% (95% CI: 0.3-4.0; 3/212}. These are reported in Table 1.

Table 1 : Anti-Zika virus Antibody status In Nairobi, Eldoret and Kisumu

Study Area	Type of Sample	Anti-zika virus antibodies status (ELISA)			
		Positive % (n)	Equivocal % (n)	Negative % (n)	Total
Eldoret	RBTC**	0.0%(0/135)	0.7%(1/135)	99.3%(134/135)	135
Kisumu	RBTC**	1.5%(2/134)	0.0%(0/134)	98.5%(132/134)	134
Nairobi	RBTC**	0.0%(0/96)	5.2%(5/96)	94.8%(91/96)	96
	SWOP*	1.4%(3/212)	0.0%(0/212)	98.6%(209/212)	212
Total		0.9%(5/577)	1.0%(6/577)	98.0%(566/577)	577

SWOP: Sex Workers Outreach Program;RBTC: Regional Blood Transfusion Centres*

All samples tested positive (n=5) and equivocal (n=6) by ELISA returned negative results by MNT, though one sample was positive at 1:80,1:160,1:320,and 1:640 serum dilutions and was negative at 1:20,1:40 and 1:1280 serum dilutions. The same sample returned a positive result at 1:20 and 1:80 serum dilutions to Zika virus {0.17 %(95% CI: 0.004-0.961; 1/577} by ZIKV PRNT and was negative to DENV (Dengue virus). Similarly, 3 samples tested positive by ELISA returned positive results to DENV at 1:20 and 1:80 serum dilutions as confirmed by DENV PRNT and were all negative to ZIKV. These are illustrated in Figure 11.

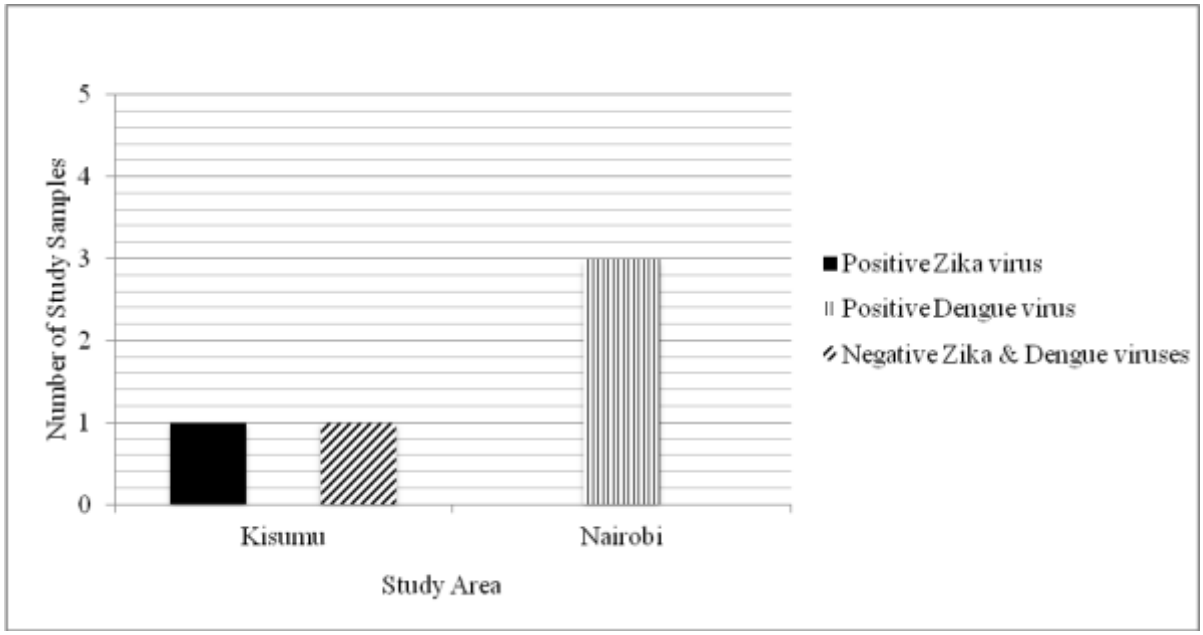

Figure 11: PRNT results of five sera tested positive by ELISA across study areas

4.2 DATA ANALYSIS

Chi-square and Fisher's exact test analysis was conducted to determine any association between the prevalence of anti-Zika virus antibodies and the study locations where the samples were initially obtained (Table 2).

Table 2: Link between study locations and prevalence of anti-Zika virus antibodies

Study Location	**Anti-Zika virus antibodies status(PRNT)**	
	Positive(*)	**Negative(*)**
Eldoret	0(0.2)	135(134.8)
Kisumu	1(0.2)	133(133.8)
Nairobi	0(0.5)	308(307.5)
Total	1(1.0)	576(576.0)
Person Chi2(2)	**=3.3117**	**P value=0.191**
Fisher's exact		**P value=0.232**

(*): expected frequency

As evidenced by Fisher's exact test analysis (P value=0.232), there was no statistical association between the prevalence of anti-Zika virus antibodies and the study locations where the serum samples were originally derived (Table 2).

CHAPTER FIVE

5.0 DISCUSSION

This study estimated seroprevalence of anti-Zika virus antibodies (IgG) in archived human sera originally obtained from three different sites in Kenya. Only one sample had anti-Zika virus antibodies (a prevalence of 0.2 %; 1/577).

Findings of this study closely reflect low circulation of anti-Zika virus antibodies as described by Sudanian, Ethiopian and Ugandan studies. One sample in Sudanian study (Zika virus-specific IgG ELISA and PRNT) tested positive for anti-Zika virus antibodies out of 845 human sera. This study utilised subset of samples obtained during 2012 nationwide yellow fever cross-sectional community based study and its findings evidenced pre-existence of Zika virus in Sudan (Soghaier *et al.*, 2018). There were 7 sera out of 112 tested positive for anti-Zika virus antibodies among other flaviviruses in a countrywide yellow fever surveillance study collected from May to July 2014 in Ethiopia (Yellow fever-specific IgG ELISA and PRNT). This indicated previous exposure of some Ethiopians to Zika virus (Mengesha Tsegaye *et al.*, 2018). Three serum samples in Ugandan hospital based arboviral surveillance study (Zika virus-specific IgM and PRNT) out of 384 were positive for Zika virus among other arboviruses. This study utilised acute febrile patient sera at St.Francis Hospital in Nkonkonjeru obtained from February 2014 to October 2017 in Uganda. This study implicated the association of Zika virus infection with febrile illness in Uganda (Crabtree *et al.*, 2018).

The positive sample by this study may indicate local previous exposure to Zika virus in Kenya. However, archived serum samples utilised in this study did not have demographical data; hence definitive confirmation of local previous exposure to zika virus is uncertain. This is because the blood donor for the positive sera from Kisumu might either be a Kenyan resident, a foreigner visiting Kenya or a Kenyan having travelled abroad.

Furthermore, utilization of archived serum samples by this study, which were conveniently retrieved from the deep freezers, may limit its comparisons with other studies conducted using other designs.

Moreover, the positive sample to Zika virus in this study was based on the PRNT results. Though, MNT and PRNT are expected to give comparable and similar results, on some occasions, the two tests may give dissimilar results. However, PRNT is widely used for ZIKV confirmation tests and its laboratory results were adopted by this study over the outcome of MNT (who, 2007; Putnak *et al.*, 2008; Roehrig, Hombach and Barrett, 2008).

Cross reaction of non-neutralizing antibodies between dengue virus and Zika virus in this study were differentiated by PRNT (Vorndam and Beltran, 2002; Putnak *et al.*, 2008). Further cross reaction of anti-zika virus antibodies with other flaviviruses such as West Nile virus and yellow fever virus were not performed and one of them may be related to the sample that was negative to both ZIKV and DENV PRNTs.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The positive serum sample obtained in 2013 from Kisumu County by this study demonstrates evidence of low pre-existing immunity to Zika virus in Kenya. However, ZIKV is a re-emerging virus and limited data of its seroprevalence in Africa, necessitates further updates of seroprevalence of Zika virus data in Kenya and other African countries through surveillance studies. This shall timely inform concerned healthcare authorities in the respective countries in Africa to make a definitive decision on what to do thereafter.

6.2 RECOMMENDATIONS

There has been low exposure to Zika virus by general Kenyan populace evidenced by this study, therefore:

- More studies should be done to know causes of low exposure of general Kenyan population to Zika virus.
- Co-ordinated regular surveillance studies backed up by confirmation tests in Kenya are needed to avail timely information for management of Zika virus in the country.

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CHAPTER SEVEN

7.0 APPENDICES

7.1 LABORATORY REPORT FORMS

STUDY TITLE: **SEROPREVELANCE OF ZIKA VIRUS IN SELECTED REGIONS OF KENYA.**

Demographic data from study participants:

Study number ……..

Date at collection of sample ……

Region of sample collection …….

TEST: IgG ELISA

RESULTS:

Positive ☐

Negative ☐

TEST: MICRONEUTRALIZATION TEST/PRNT

RESULTS:

Positive ☐

Negative ☐

7.2 NATIONAL RESEARCH FUND AWARD LETTER

National Research Fund

Telephone: +254-20-4403386
E-mail: secretariat@researchfund.go.ke
Website: www.researchfund.go.ke

Utalii House, 9th Floor;
P.O. Box 26036-00100,
NAIROBI - KENYA

Ref: NRF/1/MSc/158

20th December 2017

Bramwel Kisuya

University of Nairobi

PO BOX 30197-00100

NAIROBI

RE: AWARD OF MASTERS RESEARCH GRANT

I'm pleased to inform you that, you have been awarded a research grant for your research Masters Research project.

National Research Fund (NRF) has approved a grant of **KES 345,700** for the project titled *"Seroprevelence of Zika Virus and other arboviral infections in selected regions in Kenya"* This project is for a period of One year.

The project will be carried out in strict adherence to the guidelines detailed in NRF grant Contract. You are expected to sign the grant contract with your University in order to access the funds.

I take this opportunity to congratulate and wish you the best in your research work.

Dr. Jemimah Onsare
Ag. CEO

Copy; Vice Chancellor
UoN

7.3 KNH-UoN ERC APPROVAL LETTER

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
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Tel:(254-020) 2726300 Ext 44355

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Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
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KENYATTA NATIONAL HOSPITAL APPROVED 08 JUN 2016 KNH/UoN-ERC P.O. Box 20723-00202 NRB.

Ref: KNH-ERC/A/197

8th June, 2016

Bramuel Kisuya
Reg. No.H56/81949/2015
Dept.of Medical Microbiology
School of Medicine
College of Health Sciences
University of Nairobi

Dear Bramuel

REVISED RESEARCH PROPOSAL- SEROPREVALENCE OF ZIKA VIRUS INFECTIONS IN SELECTED REGIONS OF KENYA (P307/04/2016)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH-UoN ERC) has reviewed and **approved** your above proposal. The approval period is from 8th June 2016 – 7th June 2017.

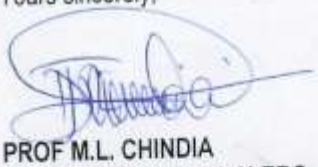
This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Protect to Discover

Yours sincerely,



PROF M.L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director, CS, KNH
The Assistant Director, Health Information, KNH
The Chair, KNH- UoN ERC
The Dean, School of Medicine,UoN
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