IgG-ANTIBODY SEROPREVALENCE OF WEST NILE VIRUS AMONG BLOOD DONORS IN NAIROBI AND NAKURU REGIONAL BLOOD TRANSFUSION TESTING CENTRES IN KENYA

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IgG-Antibody Seroprevalence of West Nile Virus among Blood Donors in Nairobi and Nakuru Regional Blood Transfusion Testing Centres in Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

I dedicate this thesis to my family, relatives and friends.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABBREVIATION AND ACRONYMS

BTS – Blood Transfusion Service

CDC – Centre for Disease Control

DNA – Deoxyribonucleic acid

ELISA- Enzyme Linked Immunosorbent Assay

FDA – Food and Drug Administration

HBsAg – Hepatitis B Antigen

HBV – Hepatitis B

HCV – Hepatitis C

HIV – Human Immunodeficiency Virus

IgG - Immunoglobulin G

IgM- Immunoglobulin M

KNBTS – Kenya National Blood Transfusion Services

NIAID – National Institute of Allergy and Infectious Diseases

PRNT - Plaque reduction neutralization test

RBTTCs – Regional Blood Transfusion Testing Centres

RNA– Ribonucleic acid

SARS - Severe Acute Respiratory Syndrome

TTI- Transfusion transmissible infection

WNV– West Nile Virus

ABSTRACT

West Nile Virus (WNV) was first discovered in Africa in 1937 and is transmitted by mosquitoes mainly of the Culex species. It can also be spread through direct contact with the blood or tissue(s) of infected animals. The natural hosts for WNV are the birds. Infection with the virus is associated with serious neurological disorders in man. Twenty percent of people who develop illness have relatively mild disease with symptoms that include fever, headache, body aches, nausea and vomiting as well as swollen glands, skin rash on the chest, stomach and back. Symptoms may be experienced for a few days or weeks or even months while over 80% of those infected may not show any symptoms. In rare cases WNV results to encephalitis leading to death. There is no known vaccine against WNV virus for humans. The main objective of this study was to determine the sero-prevalence of WNV among blood donors in Nairobi and Nakuru Regional blood transfusion testing centers in Kenya. To achieve this objective, a cross sectional study was carried out in two Regional Blood Transfusion Centers (RBTCs) which are based in Nairobi and Nakuru. These two centers were associated with possible low and high prevalence respectively. A total of 180 samples were collected and tested for other blood transmitted infections including WNV using ELISA. A data collection tool was used and analysis was done using SPSS software version 21. This study evaluated donors attended to in facilities within the lake basin versus the capital city which is highly cosmopolitan and devoid of malaria. It was therefore anticipated that some donors are likely to be harboring WNV especially in Nairobi due to its cosmopolitan nature. Majority of the donors were below 35 years of age and were predominantly male. Blood samples that were TTI positive were 40% of which 81.9% were HIV positive. WNV IgG prevalence was 15% in blood donors (95% CI 10-20.5%). Prevalence of cross infection of TTI and WNV was 8.3% (95% CI 4.4-1 2.2%). The prevalence of WVN IgG was highest in the 19-35 years age group (16.5%) and females (21.6%) though the results were not statistically significant. There was no difference in the IgG positivity between the different centers. In conclusion, infection with WNV should be of public health concern because about a fifth of those infected with WNV develop illness.

CHAPTER ONE

INTRODUCTION

1.1 Back Ground of Study

Blood transfusion is a medical procedure where a patient receives whole blood or one of the components of blood such as red blood cells, white blood cells, platelets, and clotting factors through an intravenous line. A certain volume of blood is taken from a healthy person and transfused into a patient. After the donated blood has been screened and is considered free of Transfusion Transmissible Infections (TTIs), the process of cross match begins where blood from the donor is cross matched with patients serum in a 4 step procedure (ALB 37,Coombs 37,RT 37, S 37). With confirmation, macro and microscopically, that its compatible, the blood is ready for transfusion with keen observation by the doctor of any allergic reactions using an observation chart (Patil *et al*., 2015).

1.2 Blood Transfusion in Disease Management

The first successful blood transfusion was carried out in England in the year 1665 (Rivera *et al.*, 2005). About forty years earlier in 1628, the English physician, William Harvey, had discovered the properties of blood and described its circulation but was unable to achieve success in its transfusion. The first blood transfusion from animal to human was administered by Dr. Denys on June 15, 1667. A 15 year old boy was transfused with blood of a sheep and he survived. Dr. Denys later on performed another transfusion on a laborer who survived the transfusion with no allergic reaction being noted. Blood transfusion was greatly improved and made

safer with the discovery of the A, B and O blood groups by Karl Landsteiner in 1901 (Giangrande *et al.*,2001) The grouping of patient and donor blood and cross matching soon became standard practice. Later in the 20th Century, there was development of blood banks. United States army officer Oswald Hope Robertson is generally credited with establishing the first blood bank in 1917 (Erhabor & Adias, 2013).

Blood transfusions can treat an illness and can as well cure diseases. Treatment can be for a sickness, medical condition and chronic or non-chronic disease.

Anemia results from red blood cells or hemoglobin decrease in the blood thus reduced uptake of oxygen in the circulation system. This condition is mostly managed by blood transfusion. Chronic renal failure is described as progressive loss in renal function over a period of several months or years and is highly managed by blood transfusion. Neonatal jaundice is also termed as hyperbilirubinemia, which is a yellowish/discoloration of the white part of the eyes, and skin in newborn baby due to increased bilirubin levels in blood and is managed by blood exchange/blood transfusion. Other conditions managed by blood transfusion include iron deficiency anemia and placental abruption (Patil *et al.*, 2015).

Before any blood transfusion is done, the blood should be considered safe for transfusion. In essence the blood must have been screened and considered free from TTIs such as HIV, Hepatitis B, Hepatitis C and Syphilis. Quality Assurance in blood transfusion is best achieved by: Patient identification for example, by their unique IP numbers, last and first names and age; Good Documentation: reason for blood transfusion both clinical and laboratory data and summary of information provided to

patient (for example, risks, benefits, alternatives) and patient consent; Verbal communication between clinical staff and the laboratory risks misunderstanding or transcription error (Blood safety and Availability., 2014). Communication should be used whenever possible although urgent requests for blood should be also done by phone calls accompanied by clinical summary/laboratory request and; Laboratory documentation is very necessary, that is, what time transfusion took place, reasons, blood group, cross match results and date (Patil *et al*., 2015).

1.3 Risk of Arboviral transmission during Transfusion

Arthropod-borne viruses are characterized by a biological cycle in which the virus replicates alternately in vertebrate and haematophagus hosts such as mosquitoes, ticks and sandfly. Arthropods become infected following a blood meal on viraemic vertebrates host and remain infectious for the duration of their life (Sang & Dunster, 2001). After incubation and replication in the tissues of arthropod host tissues, the virus is then passed on to non-immune vertebrate host during subsequent feeding. Thus transmission by arthropod vectors such as mosquito and ticks are termed as arboviral infections. These arthropods infections occur mostly in warm weather since that is the time they are active. Elderly people and children are more susceptible to arboviral infections. Once the blood is infected and the infected individual donates blood, it leads to infected blood with TTIs, for example, WNV, Chikungunya (WHO, 2017).

Existence of a considerable risk for transfusion transmission of arboviruses is there and it is due to short periods of asymptomatic viraemia in populations with variable

and sometimes extremely high incidence of arboviral infections. Aside from West Nile virus, few arbovirus transfusion transmissions have been proven, mostly due to difficulties in ruling out vector-borne transmission in recipients with arbovirus disease. Nevertheless, arbovirus transfusion risk models and assessments of viraemia prevalence in blood donations indicate substantial transfusion transmission of dengue and Chikungunya viruses in epidemic areas (CDC, 2015). Many other arboviruses, several of which are importation risks in the Americas, Europe and Asia, also cause large outbreaks and threaten transfusion safety. Prevention largely depends on excluding donors from outbreak areas or implementation of highly sensitive nucleic acid amplification tests. Because of the increasing emergence of arboviral disease globally, it is prudent to prepare for both endemic and exotic arboviruses capable of producing large epidemics and subsequent transfusion transmission risk (Sang & Dunster, 2001).

Emergence, re-emergence, increased outbreak frequency and growing endemicity of some important arbovirus disease for example, Dengue fever, West Nile Virus (WNV), and Yellow Fever in Kenya is a real problem that requires more attention. Continued surveillance and control strategies need to be enhanced to deal with outbreaks. West Nile Virus and other arboviruses including Yellow fever virus, dengue virus, Chikungunya virus, Rift Valley Fever and Crimean-Congo hemorrhagic fever are the most common arboviruses in Kenya. Half of these arboviruses are mosquito borne. Factors influencing these arboviruses include: vector, virus, wild vertebrate host, humans and environmental factors (Gubler & Duane, 2002) Environmental factors have the most profound effect on the rate of

emergence of the arbovirus diseases by its alteration of the vector dynamics and ecology. WNV is caused by Culex mosquitoes and most of the environmental factors promotes feeding and breeding of mosquitoes (Sang & Dunster, 2001).

1.4 Statement of the Problem

West Nile Virus can be transmitted through blood transfusion where an infected blood donor is involved. No study has been conducted in Kenya on the presence of WNV in donated blood at the Kenya National Blood Transfusion Services (KNBTS). Furthermore, the impact of unscreened blood that may carry WNV on transfusion and on patient prognosis as well as on transmission dynamics in Kenya remains unknown. The prevalence of transfusion transmitted WNV among patients is also unknown especially since most infections are asymptomatic.

1.5 Justification

West Nile Virus is a threat to human health, thus it is essential to determine the prevalence of WNV, which does not form part of the TTIs screening in donors in Kenya. WNV cases have been reported in the entire African continent and can be transmitted during blood transfusion. There are no vaccines to prevent or medications to treat WNV in people. Fortunately, most people infected with WNV do not have symptoms therefore posing a great risk of transmission. About 1 in 5 people who are infected develop a fever and other symptoms. About 1 out of 150 infected people develop a serious, sometimes fatal, illness. The findings of this research paper may therefore be used to guide policy and guideline formulation for

blood transfusion medicine in Kenya to combat future transmission during blood transfusion.

1.6 Objectives

1.6.1 Broad objective

To determine the IgG-Ab seroprevalence of WNV among blood donors tested in Nairobi and Nakuru Regional blood transfusion centers in Kenya.

1.6.2 Specific Objective

i. To determine the risk factors associated with blood donors who test positive for WNV.
ii. To determine the seroprevalence of IgG-Ab to WNV in Nairobi and Nakuru Regional Blood Transfusion Testing Centres
iii. To determine cross-infection of routinely tested TTIs with WNV.

1.7 Hypothesis of the study

i. There is no significant difference in the IgG positivity rate of the blood samples from Nairobi and Nakuru cosmopolitan regions.
ii. There is no significant difference in the prevalence of the WNV in both Nakuru and Nairobi regions.

CHAPTER TWO

LITERATURE REVIEW

2.1 Structure of West Nile Virus

A virus is either a single stranded Ribonucleic Acid (ssRNA) or double stranded Deoxyribonucleic Acid (dsDNA). dsDNA virus encode its genes in the same kind of molecule as animals, plants, bacteria and other cellular organisms, while the other types of genomes are unique to viruses. It interesting to note that most fungal viruses have dsRNA genomes, most plant viruses have single stranded Ribonucleic Acid (ssRNA) genomes and most prokaryotic viruses have dsDNA genomes. The reasons for these distributions presumably concern diverse origins of the viruses in these very different host types. A further categorization of a virus nucleic acid can be made on the basis of whether the molecule is linear, with free 5 and 3 ends, or circular, as a result of the strand(s) being covalently closed. Some linear molecules may be in a circular conformation as a result of base pairing between complementary sequences at their ends. This applies, for example, to the DNA in hepadnavirus virions and to the RNA in influenza virions (Carter & Saunders, 2007).

Virus genomes span a large range of sizes (Figure 2.1). Porcinecircovirus (ssDNA) and hepatitis delta virus (ssRNA) each have a genome of about 1.7 kilobases (kb), while at the other end of the scale there are viruses with dsDNA genomes comprised of over 1000 kilobase pairs (kbp). The maximum size of a virus genome is subject to constraints, which vary with the genome category. As the constraints are less severe for dsDNA, all of the large virus genomes are composed of dsDNA. The largest

RNA genomes known are those of some coronaviruses, which are 33 kb of ssRNA. The largest virus genomes are larger than the smallest genomes of cellular organisms, such as some mycoplasmas (Carter & Saunders, 2007).

In the secondary and tertiary structure, as well as encoding the virus proteins to be synthesized in the infected cell, the virus genome carries additional information, such as signals for the control of gene expression. Some of this information is contained within the nucleotide sequences, while for the single-stranded genomes some of it is contained within structures formed by intramolecular base pairing. In ssDNA, complementary sequences may base pair through G–C and A–T hydrogen bonding; in ssRNA weaker G–U bonds may form in addition to G–C and A–U base pairing. Intramolecular base pairing results in regions of secondary structure with stem-loops and bulges (Carter & Saunders, 2007). WNV is an enveloped, spherical single stranded Ribonucleic acid (ssRNA)

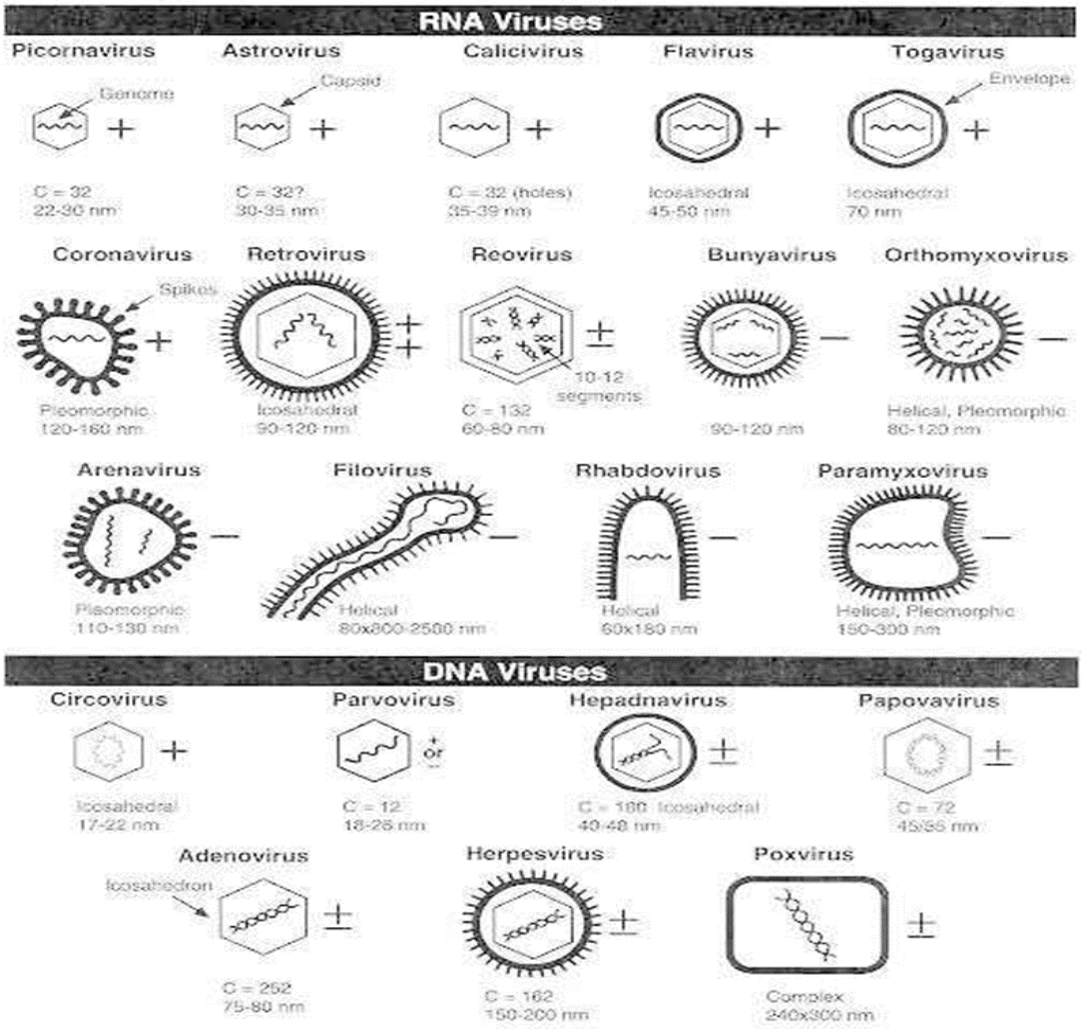

Figure 2.1: Schemes of 21 human infecting virus families showing a number of distinctive criteria.

Adapted from: Structure and Classification of Viruses medical Microbiology. 4th edition. **Legend:** (+, Sense strand; -, antisense strand; ±, dsRNA or DNA; 0, circular DNA; C, number of capsomeres or holes, where known; nm, dimensions of capsid, or envelope when present; the hexagon designates the presence of an isometric or icosahedral outline).

2.1.2 Viral Transmission Routes

A minimum proportion of the virions produced in infected hosts must be transmitted to new hosts where more virions can be manufactured in order the virus will not die out. Another way to survive is for the virus genes to be maintained in cells as nucleic acids which are replicated and passed on to daughter cells when the cells divide (Carter & Saunders, 2007). Several routes of transmission are possible for one pathogen with the transmission cycle of vector-borne infections being more complex (Figure 2.2) (Checchi, 2009). Viruses are transmitted between hosts by organisms known as vectors that feed on them. Most vectors of viruses are arthropods such as insects, mites and ticks. Arthropod-transmitted viruses of vertebrates are sometimes known as arboviruses (Carter & Saunders, 2007).

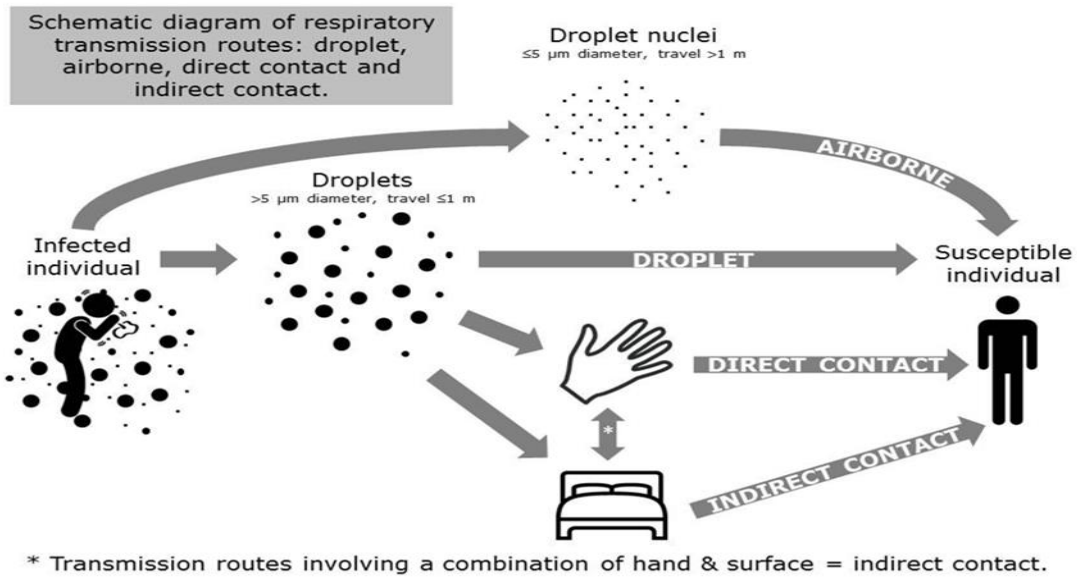

Figure 2.2: Viral Transmission Routes

The transmission cycle may also involve reservoirs such as organisms like mosquitoes.

Transmission routes are varied. It may be via air droplet with inhalation or eye contact with sneezed or coughed droplets from an infectious person, for example, cold viruses. Feacal-oral routes for diarrheal diseases like Rotavirus. Diseases like HIV and Hepatitis B may be sexually transmitted (Checchi, 2009). Wounds may also get infected with viruses such as the rabies virus (Carter & Saunders, 2007).

Vector-borne diseases where pathogens undergo a life cycle inside humans or other vectors such as insects that then transmit through bites include Malaria. Diseases can also be transmitted vertically from mother to child, for example, HIV and Hepatitis B. Blood is another way diseases like WNV, HIV, Hepatitis C and Hepatitis B are transmitted through transfusions with unsafe blood (Checchi, 2009).

2.1.3 Classification of Viruses

Viruses are grouped on the basis of size and shape, chemical composition and structure of the genome, and mode of replication. Helical morphology is seen in nucleocapsids of many filamentous and pleomorphic viruses. Helical nucleocapsids consist of a helical array of capsid proteins wrapped around a helical filament of nucleic acid. Icosahedral morphology is characteristic of the nucleocapsids of many “spherical” viruses. The number and arrangement of the morphologic subunits of the icosahedron are useful in identification and classification. Many viruses also have an outer envelope (Gelderblom, 1996).

The genome of a virus may consist of DNA or RNA, which may be single stranded (ss) or double stranded (ds), linear or circular. The entire genome may occupy either one nucleic acid molecule (monopartite genome) or several nucleic acid segments (multipartite genome). The different types of genome necessitate different replication strategies (Gelderblom, 1996).

2.1.4 Flaviviruses

The Flaviviridae family consists of positive, single-stranded enveloped RNA viruses. They are found in arthropods, that is, mainly ticks and mosquitoes. Members of this family belong to a single genus, Flavivirus. They cause widespread morbidity and mortality throughout the world. Flaviviruses transmitted by ticks are responsible of encephalitis and hemorrhagic disease, for example, Tick-borne Encephalitis and Alkhurma disease. Some of the mosquito-transmitted viruses include yellow fever, dengue fever, Japanese encephalitis, Zika virus and West Nile viruses (CDC, 2017).

2.1.5 West Nile Virus

In 1937, the first case of WNV was identified in East Africa in a female Ugandan patient after which the virus has since spread continentally to Australia, USA, Europe and Asia. Its spread has been linked to ecological factors, population growth, inadequate vector control and climatic changes. South Africa has reported WNV to cause severe encephalitis in man. It was not until 1999 when the first case of WNV was detected in North America (Cho & Diamond, 2012). Africa, Asia and Europe are among the regions where WNV is known to be indigenous (Lwande *et al.*, 2013). Within the tropical regions, the transmission of WNV increases during the rainy

season following the increase of mosquitoes. In the temperate zones, most incidences occur in summer or the beginning of autumn (Sue *et al.,* 2010). Most people involved in outdoor activities in the affected regions are more exposed and at risk of infection with the virus. WNV has no known vaccine (Cho & Diamond, 2012).

West Nile virus has an extensive distribution throughout Africa, the Middle East, southern Europe, western Russia, south western Asia, and Australia (Kunjin subtype of West Nile virus), which derives from its ability to infect numerous mosquito and bird species. Until the early 1990s, human outbreaks, mainly associated with mild febrile illnesses, were reported infrequently from Israel and Africa. Since then, new viral strains with likely African origin have increased human disease incidence in parts of Russia and southern and eastern Europe, with large outbreaks of increased clinical severity occurring in Romania, Russia, Israel, and Greece. Although West Nile virus now circulates in many countries in the western hemisphere, for unknown reasons only the United States and Canada have experienced substantial human disease incidence (Petersen *et al.,* 2013).

Ornithodorosmoubata, a species of tick is also reported to be one of the vectors for the virus. The major vector is reported to be mosquitoes of the Culex species. These include Culex thriambus, Culex tarsalis, Culex pipens, and Culex nigripalpus among others. However, over sixty five mosquito species are said to have contributed to the spread of the infection with various vectors contributing to spread to specific hosts. Birds including ravens, crows and jays are documented to have been major contributors of the viruses' spread to Europe and Middle East (Lwande *et al.*, 2013).

West Nile Virus (WNV) is transmitted by infected mosquitoes which cause most of its incidence. Mosquitoes pick the virus after feeding on infected birds which then lead to transmission to humans through bites (CDC, 2015). Birds are known to be the natural hosts of WNV while humans are the incidental hosts. As a result there is a mosquito-bird-mosquito cycle for WNV which consequently results to human infection. The Culex species of mosquitoes is primarily involved in the spread of WNV (Sue *et al.,* 2010). Other species such as Aedes and Anopheles may be involved in the spread of the virus (Lwande *et al.,* 2013)

WNV can also be transmitted through blood transfusion where an infected blood transmitter is involved (Sharifi *et al.,* 2010). Transmission may also occur during organ transplants and during delivery, pregnancy or breastfeeding from mother to child (Petersen *et al.,* 2013).

Most patients with West Nile virus–related illnesses are unrecognized clinically. In U.S. a follow-up study of asymptomatic, viremic blood donors who subsequently developed West Nile fever showed that 38% sought medical care and 2% were hospitalized for symptoms attributable to the infection. However, only 5% of those seeking medical care were correctly diagnosed. Thus, incidence trends are best monitored by the incidence of neuroinvasive disease because reporting of these cases is comparatively complete. Nevertheless, even during a well-publicized outbreak, only 40% of patients with clinically compatible meningitis or encephalitis were tested for West Nile virus (Petersen *et al.,* 2013).

A total of 16 196 patients with West Nile virus neuroinvasive disease with onsets from 1999 through 2012 and 1549 deaths have been recorded in the United States. Although the number of patients with neuroinvasive disease fluctuates annually, some regions experience persistently higher incidence. Ninety-four percent of patients with West Nile virus infection have symptom onsets in July through September. Extrapolations from neuroinvasive disease case reporting in the United States suggest that through 2010 approximately 3 million persons were infected, of whom 780 000 developed West Nile fever. In Canada, West Nile virus was first detected in southern Ontario in 2001 and by 2009 the virus' distribution had extended west ward to British Columbia. Through October 2012, 975 patients with neuroinvasive disease have been reported in Canada (Petersen *et al*., 2013).

2.1.5.1 Transmission of WNV

Culex mosquitoes are the main vectors responsible for the spread of WNV (Koka *et al.,* 2011) WNVS states that the most predominant means of WNV transmission is via mosquitoes. It has been noted that laboratory acquired infections can be airborne or percutaneous inoculation. (Cho & Diamond, 2012)

Transmission starts when a mosquito bites an infected enzootic bird or an animal and gets the virus while feeding on the animal's blood. The infected mosquito can then transmit the virus to another bird or animal when it feeds again (Figure 2.3).

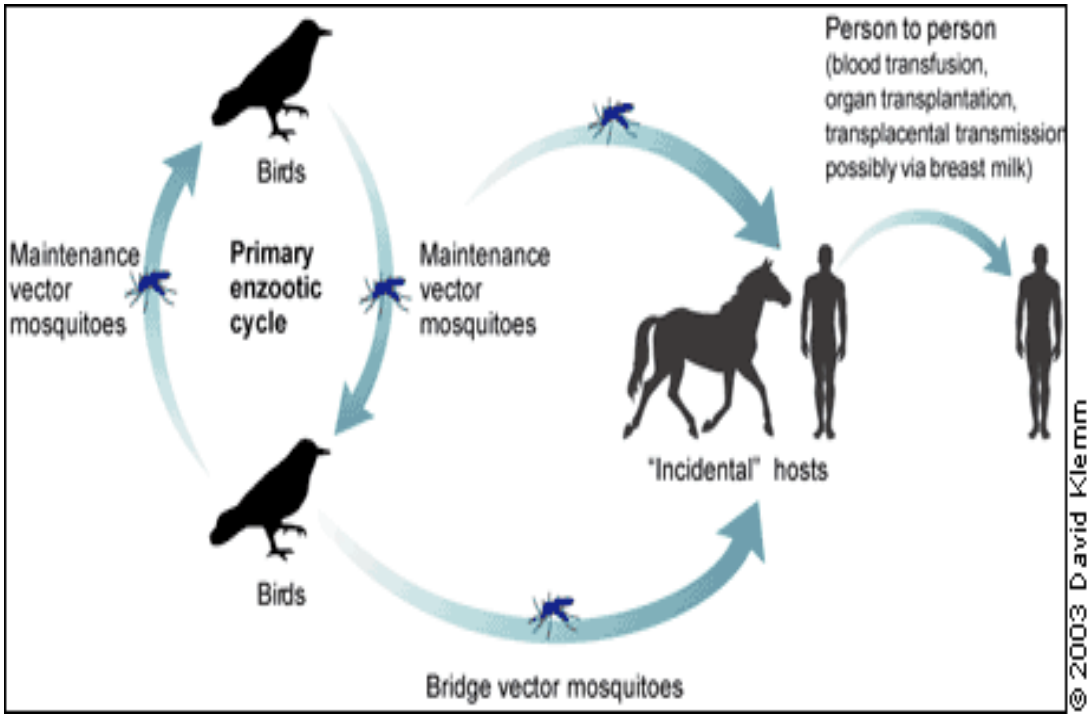

Figure 2.3: WNV Transmission Cycle.

Source: CDC National Center for Emerging and Zoonotic Infectious Diseases, Division of Vector-Borne Diseases.

Mosquito bites account for nearly all human infections. West Nile virus can also be transmitted via transfused platelets, red blood cells, and fresh frozen plasma as well as through heart, liver, lung, and kidney transplants. Transmission via organ transplant has occurred from donors without detectable viremia, suggesting viral sequestration in organs shortly after viremia has cleared. One possible transplacental transmission following a second trimester infection resulted in an infant with chorioretinitis, lissencephaly, and cerebral white matter loss. Fortunately, fetal abnormalities due to intrauterine infection are uncommon with none of 72 live infants born to 71 women infected during pregnancy had malformations linked to

WNV. Nevertheless, 3 neonates born to women infected within 3 weeks' prepartum developed symptomatic West Nile virus disease at or shortly after birth, indicating the possibility of intrauterine infection or infection at the time of delivery. Other rare or suspected modes of transmission include breast milk transmission, percutaneous or conjunctival exposure to laboratory workers, and by unknown means in patients undergoing dialysis and workers at a turkey breeder farm (Petersen *et al.,* 2013).

Scientists have identified more than 138 species of bird that can be infected with WNV and more than 43 mosquito species that can transmit the virus. Most cases of human disease occur in people over 50 years of age and in people with impaired immune systems. In a number of cases WNV has been spread through blood transfusion, organ transplant and breastfeeding. (Cho & Diamond, 2012)

2.1.5.2 Pathogenesis

The virus is spread by infected mosquitoes that bite humans (Figure 2.4). However, few cases of its spread by blood transfusion have been documented. Organ donation may also cause the spread of WNV (Ceccaldi *et al.,* 2004).

People who are at high risk of WNV acquisition are those involved in outdoor activities. Individuals over sixty years of age and those with other medical conditions such as cancer and kidney diseases are at more risk of severe disease following infection by WNV (Kuno *et al.,* 2001).

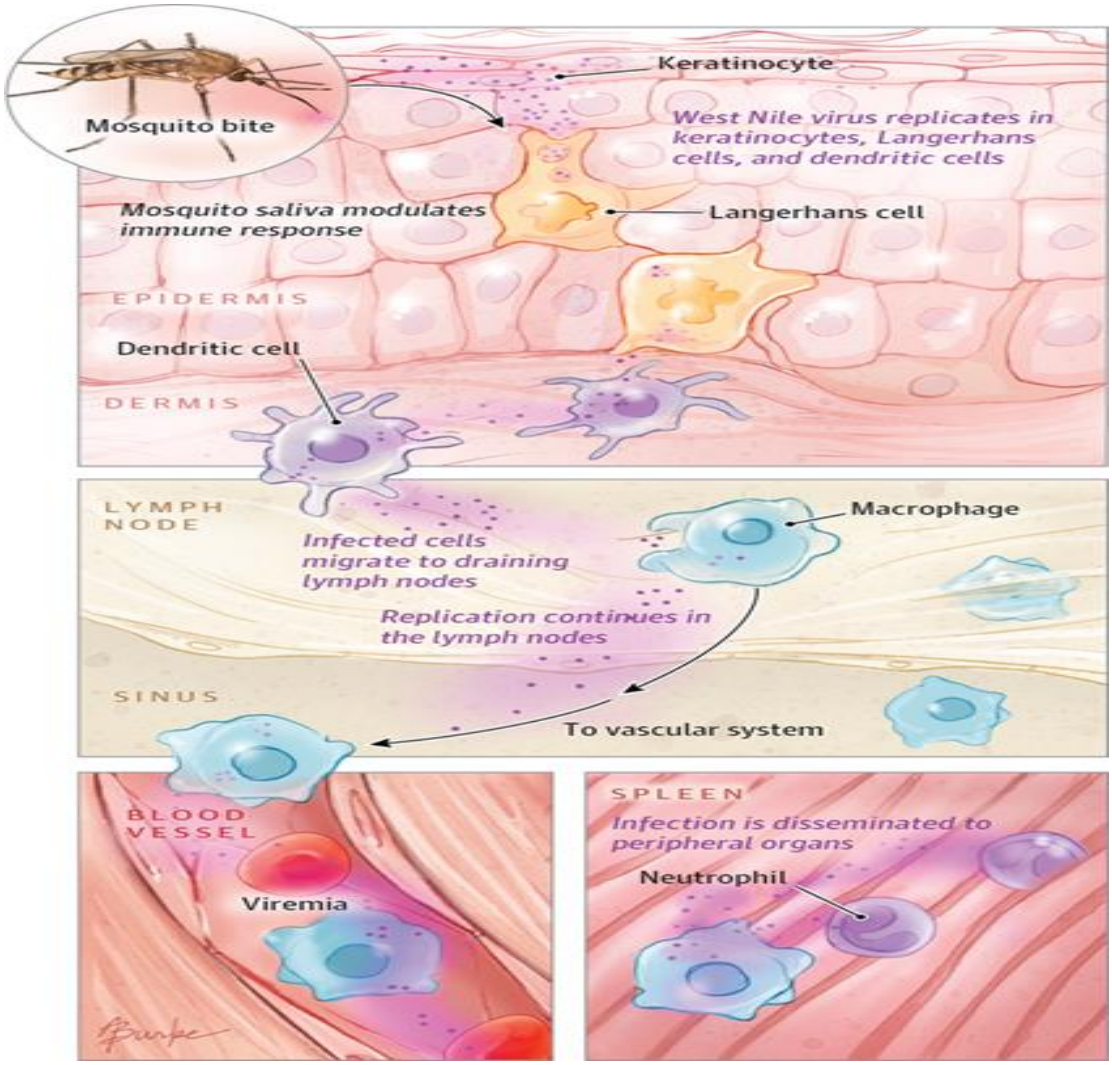

Figure 2.4: Schematic WNV Pathogenesis

The incubation period of WNV ranges from two to fourteen days based on medical conditions for the infected patients (Petersen *et al.*, 2013).

2.1.5.3 Clinical Disease

Approximately 80% of people who are infected with WNV will not show any symptoms at all. 20% of people who develop illness have relatively mild disease with symptoms that include fever, headache, body aches, nausea and vomiting as

well as swollen glands, skin rash on the chest, stomach and back. Symptoms may be experienced for a few days sometimes for weeks or even months (CDC, 2015).

About 1 in 150 people infected with WNV will develop severe illness with severe symptoms such as high fever, headache, neck stiffness, stupor, disorientation, coma, tremors, convulsions, muscle weakness, vision loss, numbness and paralysis. Serious illness can occur in people of any age: however people over age 50 and those with weakened immune function area at a highest risk for getting severely ill when infected with WNV. These symptoms may last several weeks to years and neurological effects may be permanent according to CDC. Less than 1% of those infected have severe symptoms such as meningitis and encephalitis. Out of five infected people, one suffers from headaches, fever, aching joints, diarrhea among others. About seventy to eighty percent of those infected by the virus do not develop any symptoms. Approximately ten percent of those who develop neurological symptoms succumb to the disease. Some people may develop permanent defects from the same. People typically develop symptoms between 3 and 14 days after being bitten by an infected mosquito (CDC, 2015).

It is not known what proportion of persons develop West Nile virus infection following an infected mosquito bite. Persons with a genetic defect in the OAS1 gene (HGNC 8086), which modulates host response to exogenous viral RNA, are more likely to have anti–West Nile virus antibodies than persons without this defect, suggesting that immune response function determines who becomes infected after exposure. Among persons who become infected, approximately 25% develop West Nile fever and 1 in 150 to 250 develops neuroinvasive disease. Risk factors for

developing West Nile fever following infection are poorly defined. A follow-up study of asymptomatic, viremic blood donors indicated that increasing viral load and female sex, but not age, subsequently increased the risk of developing West Nile fever. A smaller follow-up study of viremic blood donors suggested that younger persons were more likely to develop West Nile fever. In contrast, advancing age profoundly increases the risk of neuroinvasive disease, particularly encephalitis. The risk may approach 1 in 50 among persons aged at least 65 years, a rate 16 times higher than that for persons aged 16 to 24 years. Also, a history of cancer, diabetes, hypertension, alcohol abuse, renal disease, and chemokine receptor CCR5 deficiency as well as male sex may increase the risk of neuroinvasive disease. Persons infected through transplant of infected organs are at extreme risk of developing neuroinvasive disease though conflicting data exist regarding risk among previous organ recipients infected via mosquito bite (Petersen *et al.,* 2013).

2.1.5.4 Diagnosis

The diagnosis of WNV is based on clinical signs and symptoms. Serology is the most used laboratory tests in diagnosis of WNV. Laboratory tests are conducted for both the blood and spinal fluid to detect antibodies formed by the immune systems to fight WNV. The tests are done to determine abnormal immunoglobulin production. In turn the doctor is able to know whether an individual has been exposed to an antigen. The presence of IgM in the body's serum or cerebrospinal fluid is an indication of a recent WNV infection. Similarly, the detection of IgG in the serum of a patient implies post exposure of WNV (West Nile Virus Sero).

Apart from common ELISA test for WNV, other tests may include viral cultures and tests to detect the viral RNA being done on the serum, CSF and tissue specimen. This specimen is collected in the early stages of illness and an infection is confirmed by a positive test result.

Another test that can be performed is immunohistochemistry which detects WNV antigen in formalin-fixed tissue. A negative test result for WNV from the above tests may not be a confirmatory test for the lack of WNV Disease (CDC, 2015).

2.1.5.5 Prevention of West Nile Virus

West Nile virus prevention relies in part on methods to reduce the numbers of West Nile virus–infected mosquitoes. Community-based mosquito control programs using integrated pest management principles proactively identify the sources of vector mosquitoes and use several methods such as elimination of breeding sites, larviciding, and targeted adult mosquito control to prevent adult mosquito populations from achieving levels that increase human infection risk.

When increasing human case incidence or surveillance of vector mosquito populations indicates the possibility of a human epidemic, the immediate goal is to reduce rapidly the number of infected adult mosquitoes by widespread ultra-low volume application of organophosphate or synthetic pyrethroid insecticides (Petersen *et al.,* 2013). The spread of WNV can also be prevented by taking the following strict measure; preventing mosquito bites by the use of mosquito's nets and repellants and by wearing protective clothes with long sleeves. Additionally, bush clearing and

emptying standing water is necessary to prevent breeding. Screen windows and doors can be used to keep mosquitoes out (CDC, 2015).

About 10% of those who develop neurological symptoms succumb to the disease. There are also no medications to treat or vaccines to be used in the prevention of WNV. Further testing is recommended for all positive samples for confirmation as this is outside the scope of this study. Some symptoms, for example, fever can be lowered by over-the counter pain relievers. Patients may recover after months or weeks based on the severity of the disease (CDC, 2015).

2.1.5.6 Treatment of West Nile Virus.

Treatment of West Nile virus infection remains supportive. Several investigated therapeutic approaches include immune γ-globulin, West Nile virus–specific neutralizing monoclonal antibodies, corticosteroids, ribavirin, interferon α-2b, and antisense oligomers. However, no study has documented efficacy, in part due to difficulty in recruiting sufficient numbers of patients. Case reports or uncontrolled clinical series suggesting efficacy should be interpreted with extreme caution due to West Nile virus's highly variable clinical course (Petersen *et al.,* 2013).

2.1.5.7 West Nile Virus Immunity

Humoral immunity is an essential aspect of immune system-mediated protection from WNV. B-cell-deficient mice uniformly died after WNV infection but were protected by passive transfer of immune sera. Mice developed high viral loads in all tested tissues and demonstrated complete lethality after WNV infection. In prospective studies, the level of WNV-specific IgM at day 4 after infection predicted

disease outcome. While it is apparent from passive-transfer studies that immune IgG can protect against flavivirus infection, the function of IgG during primary infection is less clear. (Wang *et al.*, 2004). In mice, WNV-specific IgG is not produced until somewhat late in infection (days 6 to 8), after both viral seeding of the CNS and clearance from peripheral tissues have occurred. Thus, while it is possible that WNV-specific IgG alters WNV infection of the CNS, current data suggest that by the time IgG is produced, the survival of the animal may already have been largely determined. (Wang *et al*., 2009).

The majority of neutralizing antibodies are directed against regions of the WNV E protein, although a subset likely recognizes the prM protein. The E protein has three structural domains that mediate viral attachment, entry, and viral assembly and elicit antibodies with distinct neutralization potentials. The putative receptor binding domain, DII encodes the fusion loop involved in pH-dependent fusion of virus and host cell membranes, and DI participates in E-protein structural rearrangements required for fusion. Crystallography, nuclear magnetic resonance, and epitope-mapping studies have shown that E-specific neutralizing antibodies map to all three domains. Human single-chain variable-region antibody fragments against DII were protective in mice when administered prior to and after WNV infection. However, the most potent inhibitory antibodies recognize a dominant neutralizing epitope on the lateral face of DIII Neutralizing antibodies to this DIII epitope were detected in serum from WNV-convalescent patients and protected mice even when administered after WNV had spread to the CNS (Wang *et al.*, 2004).

Although neutralizing antibodies generated during WNV infection predominantly bind structural proteins, antibodies to the nonstructural protein NS1 also protect mice against WNV infection. NS1 is a highly conserved glycoprotein that is not packaged within the virus but is secreted at high levels from infected cells and associates with cell surface membranes through undefined mechanisms. NS1 is a cofactor in replication and is detected in the serum of infected animals during the acute phase of WNV disease. Although the function of soluble NS1 in WNV pathogenesis is not well understood, a recent study suggests that NS1 may inhibit complement activation by binding regulatory protein factor (Wang *et al*., 2009).

Experiments with small-animal models have demonstrated that T lymphocytes are an essential component of protection against WNV. Consistent with this, individuals with hematologic malignancies and impaired T-cell function have an increased risk of neuroinvasive WNV infection. Upon recognition of a WNV-infected cell that expresses class I MHC molecules, antigen-restricted cytotoxic T lymphocytes proliferate, release proinflammatory cytokines, and lyse cells directly through the delivery of perforin and granzymes A and B or via Fas-Fas ligand interactions. Mice deficient in CD8 T cells or class I MHC molecules had normal humoral responses but greater and sustained WNV burdens in the spleen and CNS and increased mortality. Granzymes appear to be important for control of the lineage II isolate Sarafend, with perforin, Fas, and Fas ligand having a more limited role in modulating infection. In contrast, CD8 T cells require perforin to control lineage I WNV as mice deficient in perforin molecules had increased CNS viral burdens and lethality. Moreover, adoptive transfer of wild-type but not perforin-deficient CD8 T cells

decreased CNS viral burdens and enhanced survival. CD4 T cells likely contribute to the control of infection through mul-E16 contact residues and binding of WNV virions. Preliminary data suggest that CD4 T cells restrict WNV pathogenesis in vivo. CD4 T-cell depletion or a genetic deficiency in MHC class II molecules results in attenuated WNV-specific antibody responses and increased lethality (Volk *et al.*, 2004). Although much remains to be learned about the specific mechanisms that define immunity against WNV, both humoral and cellular responses likely are essential for protection. Specific neutralizing antibodies are generated at late times after primary WNV infection and the development of high-titer neutralizing antibodies after vaccination correlates with protection against challenge. Nevertheless, it remains unknown how the epitope specificity of the antibody repertoire contributes to protective memory responses. T-cell-mediated immunity is also likely necessary to resist a second WNV challenge. T-cell-deficient mice infected with WNV had decreased memory CD8 T-cell responses and were more susceptible to secondary infection, and adoptive transfer of CD8 T cells from WNV-primed wild-type but not T-cell receptor (TCR) mice increased the survival of naive mice. Immunization with different WNV vaccine preparations can induce memory T cells, although the relative contribution of humoral versus cellular memory to vaccine efficacy remains largely undefined (Samuel & Diamond, 2006).

2.2 Blood banking and transfusion

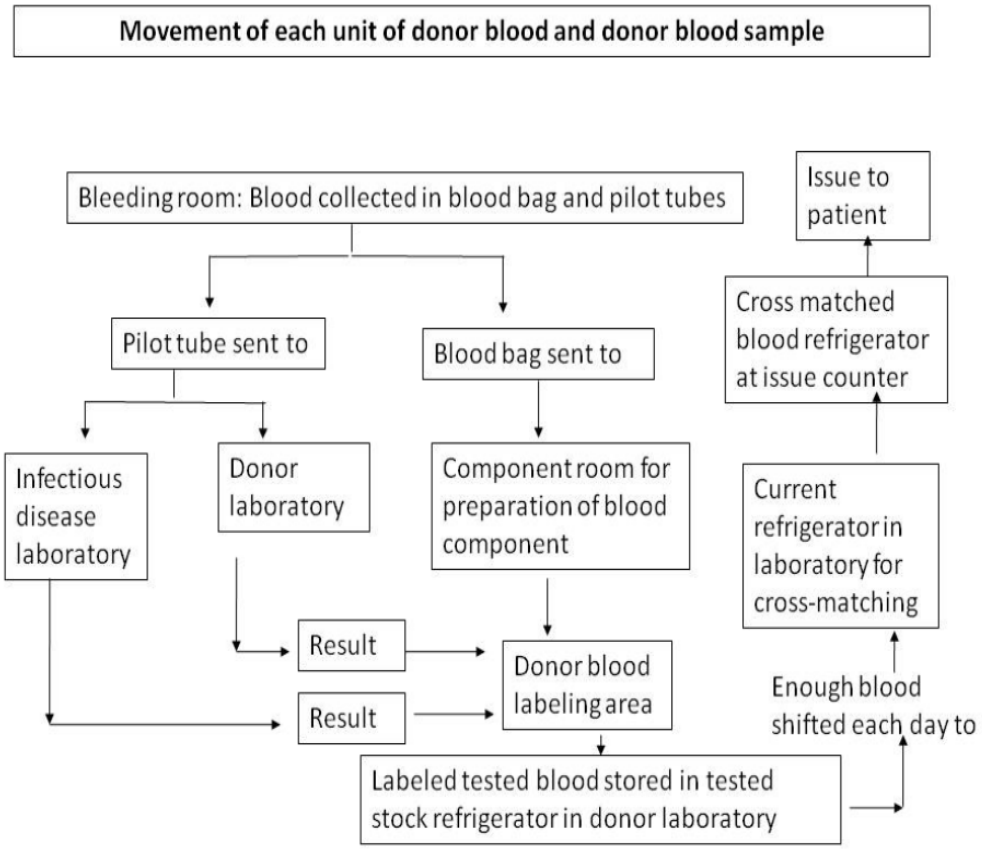

Figure 2.5: Movement of donor blood and donor blood sample.

Adopted from the National Blood Transfusion Services guide of 2008.

Blood is collected in the bleeding room and moves through different stages before it is issued to the patient as shown in Figure 2.5.

2.2.1 Blood bank safety precautions

Laboratory personnel are informed of the hazards such as transmission of viral infection when working in a blood bank laboratory. The possible routes of infection

include skin abrasion or puncture through body orifices. Incidental exposure to infected samples like bag leakage, splash and needle stick injury should be reported to the concerned authorities. Preventive inoculation of the blood bank staff against Hepatitis-B infection after appropriate tests is necessary (Boyan *et al.,* 1963). Staff should wear protective apparel and be made aware of universal precautions that include hand safety and hygiene; use of laboratory coats; not putting objects in the mouth or food in laboratory equipment; and avoiding needle pricks (Patil *et al*., 2015).

2.2.2 Blood donor recruitment and retention

Recruitment of donors is critical to the success of supply of safe and adequate blood and its product so at to meet patients need. Different strategies are used to recruit donors. They include pure voluntary-based recruitment, social persuasion-based recruitment and remuneration-based donations. The first two strategies result in voluntary blood donation as they do not rely on monitory remuneration thus the information of their health provided by these donors can be trusted. Remunerated based blood donation is done by blood sellers who may conceal the facts about their health and diseases which they carry, about their last donation and their identity (Patil *et al.,* 2015). However in Kenya, blood donation is voluntary and non-remunerated (MOH, 2001).

Pure voluntary recruitment strategy depends on the internally generated sense of community responsibility. However, most donors do not donate blood as often as they can. Social persuasion is associated with persuasion and pressure from friends,

colleagues, heads of religious organization and political leaders. In the case of remunerated-based donations, it is done by blood sellers who give blood for money.

Oral communication is the most effective method of recruiting donors. Other methods include circulars, direct mail, print and mass media, and education among youth in educational institutions on blood donation. Donor retention strategies include public image building, convenient and attractive blood collection centers (Patil *et al.*, 2015).

2.2.3 Blood group systems

A group of antigens controlled by a locus having a variable number of allelic genes is known as a blood group system. More than 15 blood group systems are recognized. Of these, the AOB system is the most important. It is the only system where if there is a lack of an antigen, there is always the corresponding antibody (Patil *et al.*, 2015). The AOB system has four main blood groups (A, B, AB and O). These blood groups are determined by the genes inherited from a person's parents. Blood group A has A antigens on the red blood cells with anti-B antibodies in the plasma while blood group B has B antigens with anti-A antibodies in the plasma. In blood group O, it has no antigens, but both anti-A and anti-B antibodies in the plasma. Blood group AB has both A and B antigens, but no antibodies. Blood group O is the most common blood group (NHS, 2017).

Anti-A and anti-B are potent hemolysins capable of causing intravascular hemolysis by bind complement to C9. Hence in blood transfusion, the ABO of the patient and the donor must be compatible (Patil *et al.,* 2015). Receiving blood from the wrong

ABO group can be life threatening. For example, if a person from blood group B is given blood that is A, their anti-A antibodies will attach the A cells. However group O red blood cells do not have A or B antigens hence can be safely given to any other group (NHS, 2017).

Red blood cells sometimes have another antigen which is a protein known as the RhD antigen. When it is present, the blood group is RhD positive and when it is absent the blood group is RhD negative. In most cases, O RhD negative blood (O-) can be safely given to anyone. This is because it does not have any A, B or RhD antigens on the surface of the cells and is therefore compatible with every other ABO and RhD blood group (NHS, 2017).

2.2.4 Preservation of blood and its components for transfusion

A designated area is used for storage to limit deterioration and prevent damage to materials in process and final products. Access to such areas is controlled. Refrigerators or freezers in which blood are stored should be used for this purpose only. Reagents are stored in separate refrigerators. Adequate alternate storage facility and written display of instructions to maintain the blood and components in the event of failure of power or equipment should be provided in the area of preservation. The alarm of all storage equipment should signal in an area that has adequate personnel coverage round the clock to ensure immediate corrective action (Boyan *et al.*, 1963).

Whole blood, red cell concentrate, should be transported in a way that will maintain a maximum temperature of 10° C ± 2 °C. Platelet/ granulocyte concentrate is stored and transported at 22°C ± 2°C. Components stored frozen should be transported in a

manner to maintain them frozen. When these are issued for transfusion, these should be thawed at 37° C prior to issue. The temperature during transport should be monitored.

Whole Blood should be stored at 40 C ±20 C in plastic blood bags. Whole blood collected in anticoagulant citrate-phosphate-dextrose solution (CPD) should have an expiry date, not exceeding 21 days after phlebotomy. Whole blood collected in anticoagulant citrate-phosphatedextrose with adenine (CPDA-1) should have an expiry date not exceeding 35 days after phlebotomy. Whole blood in heparin solution should have expiry period not exceeding 24 hours after collection.

Red blood cells which are separated in a closed system should have the same expiry date as the whole blood from which it is prepared. The time of removal of plasma is not relevant to the expiry date of red cell concentrates. However, if an open system is used, the expiry date should be 24 hours after separation. Red cell concentrate should be stored at 4° C ± 2°C. Red cells containing additive solutions such as SAGM, ADSOL, NUTRICEL should be stored up to 42 days with day of collection considered as day zero. At midnight the day is completed, for example, if platelets are separated on first of the month, expiry date should be 6th midnight. The expiry date for glycerolized (low or high) frozen red cells is 10 years and should be stored between -80°and -196° C. Washed red blood cells and deglycerolized red blood cells should be stored at 4°C ± 2°C and should be transfused as soon as possible and within 24 hours after processing. Leucocyte-poor red blood cells should be stored at 4° C± 2°C. It should have the same expiry date as whole blood from which it has been prepared, if closed system is used. In case of open system, the expiry will be

within 24 hours. The platelet concentrate should be stored between 22° C± 2°C with continuous gentle flatbed agitation (60-70/min) or a rotor (5–10 cycles/min.) maintained throughout the storage period. The expiry date of platelet concentrate prepared in a closed system should be 3 days after the collection of original blood. The expiry date may be extended to 5 days or longer when special plastic bags or anticoagulants are in use. The storage temperature for leucocyte concentrate is 22°C± 2°C. It should be transfused as soon as possible and not later than 24 hours of phlebotomy.

Single donor plasma should be separated from whole blood at any time up to 5 days after the expiry of the whole blood. The plasma separated after expiry date should be used for fractionation. If separated during shelf life, should be stored for 1 year at -30° C or lower and used as plasma for transfusion. Fresh frozen plasma and cryoprecipitate should be stored at -30° C or below and should be stored no longer than 12 months. If fresh frozen plasma remains unused at the end of one year at -30° C, it may be labelled as “plasma & used up to 5 years. If FFP is stored at -60°C or below with continuous monitoring it should be used up to 5 years. Expiry date of any component should be calculated by considering the day of collection as day zero (Patil *et al.*, 2015).

2.2.5 Transfusion transmitted infections

There are four main groups of micro-organisms that are known to cause infections. Individuals with fungal infections are usually too ill to be accepted as blood donors. Viruses, bacteria and protozoa have been reported to be transmitted in blood

transfusion. Viruses are the most common to be transmitted through blood transfusion. Some of the blood transfusion infections include:

Human Immunodeficiency virus (HIV) belongs to the retroviruses group. The HIV particles cannot be seen through an ordinary microscope but can be seen clearly with an electron microscope. Its core consists of two identical strands of RNA. Methods for HIV testing are ELISA for testing HIV p24 antigen, Polymerase Chain Reaction (PCR) and viral isolation.

Hepatitis B virus belongs to the family of hepadnaviridae. Its core consists a double stranded circular DNA and DNA polymeras. The main route of transmission involves direct contact with body fluid, for example, transfusion of infected blood or blood products. A variety of serologic markers appear following the infection with HBV, and one of these is HBsAg. This antigen appears before biological evidence of liver disease or jaundice. This persists throughout the acute phase of the disease and declines during convalescence.

Hepatitis C virus is the most common of post transfusion Non-A, Non-B hepatitis all over the world. The prevalence of HCV antibodies in blood donors in developed countries ranges from 0.4-2%. The average incubation period is 6-7 weeks, it may be as less as 2 weeks or as much as 26 weeks, the acute illness (jaundice) is mild.

Cytomegalovirus (CMV) is known to be carried in leucocytes. Blood components containing white blood cells are more likely to transmit CMV. Screening tests for CMV are latex agglutination, enzyme immunoassay and complement fixation.

Malaria is caused by intra-erythrocytic protozoan parasites, *Plasmodium vivax, P.falciparum, P.ovale or P.malariae.* The usual mode of transmission is via the bite of anopheles mosquito. Blood infected with malarial parasites can also transmit the infection. Transfusion-transmitted malaria may become evident a week to several months following the transfusion of infected blood. The incubation period of *plasmodium vivax* and *P.falciparum* is the shortest, for example, one week to one month while that of *P.malariae* is the longest and may be for many months. Screening tests for Malaria include microscopic examination of thick and thin blood smear. Tests for antibody to malarial parasites are indirect fluorescent antibody test (IFA), ELISA, immuno-diagnostic testing, including EIA methodology, and nucleic acid probe methodology, including polymerase chain reaction (PCR) (Patil *et al*., 2015).

Arthropod-borne viruses or arboviruses can be spread by mosquito vectors over great distances. Zoonotic arboviruses circulate in sylvatic and peridomestic cycles involving animals and nearby humans. Often these arboviruses remain undetected by health care systems. These arboviruses cause disease such as yellow fever, chikungunya fever, Rift Valley fever and West Nile fever (LaBeaud *et al.*, 2011).

2.3 Gaps of Knowledge and Health Concerns

The data on evolution and diversity of WNV is scarce (Lwande *et al.*, 2013). There is lack of awareness of the impact of the disease on both animals and humans. This study is intended to provide an in-depth insight on the factors that contribute to the

spread of the virus by Culex mosquito and its impact on the health of Kenyans. It is intended to contribute to lower the spread of WNV as a TTI.

2.4 Studies done on the prevalence of West Nile Virus.

West Nile virus is the leading cause of domestically acquired arboviral disease in the United States with 95% of all the cases being WNV disease (Zeller *et al.,* 2004).

This makes it important to prevent through direct activities such as screening of blood donors. Illness onset was reported during July-September. The median age of patients was 58 years (IQR 466-69) and 59% were male (Krow-Lucal *et al*., 2017).

A study of seroprevalence of WNV in New Zealand blood donors was conducted. The aim was to determine whether there was a risk of exposure for donors who had previously travelled to WNV endemic regions. The goal of the study was to produce facts with the hope of lowering the rate of transfusion- transmitted WNV. The study was conducted over a period of one year. Out of 1208 blood donor sera collected, 25 samples were positive with IgG. The prevalence of WNV in this study was 2.1% (Sue *et al.,* 2010).

The prevalence of WNV infections were reported as low in Turkey. Nineteen (1.6%) of the samples yielded WNV IgG positivity with ELISA, and all of which were IgGs with high avidities (Avidity index values were between 67.8-99.2%). Eight of 19 (42.1%) WNV ELISA IgG positive donors, had risk factors such as joining outdoor activities, contact with mosquitos and ticks and consuming raw milk and milk products (Ayturan *et al.,* 2011).

An Iranian study on the incidence and prevalence of WNV in Iranian donors was conducted using 500 samples from blood donors. The samples were examined for IgM and IgG antibodies to WNV using ELISA method. The presence of WNV RNA was tested by the real- time RT- polymerase chain reaction assay. The data was later analyzed using Chi- Square test. The results indicated that all 500 donors were negative for WNV- Specific Ig M antibody. No positive samples for WNV RNA were found. The study concluded that safety measures to lower the transmission of WNV through blood donation are a necessary precaution (Sharifi *et al.,* 2010).

A study on the epidemiology of WNV in Ghana did not detect WNV RNA but 4.8% and 27.9% but carried specific IgG in children and adults respectively. 2.4% of the children had IgM. The prevalence of IgG antibody to WNV increased progressively and peaked around 30% between the ages 1 and 30 years then stabilized. Most infections occur early in life, and as the window for infection short, the risk of transmission by transfusion appears to be low, and the risk of pathogenicity in immunocompetent recipients appears to be limited in an endemic area such as Ghana (Wang *et al.,* 2009).

A cross sectional study in Zambia reported a 10.3% WNV infection. Farmers were 20% less likely to have an infection compared to students. Participants who lived in grass roofed houses were 2.97 times more likely to be infected than those who lived in asbestos roofed houses. Travelling to Angola was associated with the infection (Mweene-Ndumba *et al.,* 2015).

Reports have indicated ongoing transmission in regions of sub-Saharan Africa. Eleven cases of acute febrile illness were caused by WVN in Guinea in 2006 while a 2009 seroprevalence study in Ghana indicated that WNV is endemic with most WNV cases occurring in childhood. In eastern Africa, human infections and mosquitoes positive for WNV lineage 2 were reported in Djibouti from 2010-2011 (Chancey *et al.,* 2015).

Some studies in Kenya have been done in relation to WNV. A study done by Tigoi *et al.,* 2015) indicated that the sero-epidemiology of selected arboviruses in febrile patients visiting selected health facilities in Lake/River basin areas of L.Baringo , L. Naivasha and Tana River in Kenya showed that the prevalence of WNV is 12.4% of 379 sample patients. It was also discovered that WNV was circulating in all the 3 sites. In a study by Muyeku (2011) the prevalence was 31% with 296 children at Alupe District hospital in Western Kenya testing positive for WNV. Mosquito-borne arbovirus surveillance was conducted at selected sites in diverse ecological zones of Kenya by Ochieng *et al.,* (2013) showed that 97% of WNV isolates obtained from samples collected from 5 pools of Culex univittatus. LaBeaud *et al.,* (2011) study of arbovirus prevalence in Mosquitoes in Kenya indicated that out of 105 Culex mosquitos' pools tested 18% were positive for WNV.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site and location

The study was conducted at the Regional Blood Transfusion Testing Centers (RBTTCS) located in Nairobi and Nakuru (Figure 3.1). Nakuru is located in the mid-western part of Kenya and serves a wide variety of people. This region serves a wide catchment area that includes towns such as: Baringo, Naivasha and Molo, all of which are known to have a contrasting variation of weather patterns. The West Nile virus is known to affect populations along the lake basin and these regions form a good representation of the target population. Nairobi is the capital city of Kenya with a population of about 5 million people. All communities are represented and the RBTC serves this population and the environs. Nairobi is a low WNV prevalent area while Nakuru is a high WNV prevalent area because of the surrounding lake basin environment. The coverage of these areas in the study was intended to limit sampling bias in high and low prevalent areas for WNV.

3.2. Study design

This was a cross- sectional descriptive study.

3.3. Study samples

Recently archived Blood (less than 7 days old) was obtained for TTI testing in the two regions of Nairobi and Nakuru. This is mainly because these two centers have been associated with possible low and high prevalence respectively.

Figure 3.1: Kenyan map showing the study areas (circled in red: Nakuru and Nairobi)

3.3.1 Inclusion criteria

Archived blood that met the following criteria were included in the study:

1. Screened for TTIs (HIV, Hep B, C and syphilis)

2. Stored for not more than 7 days old from after collection

3. If spun and unaliquoted, then they were less than 7 days old and stored at 2-8oC

3.3.2. Exclusion criteria

1. Hemolysed blood

2. Sample (kept at Room Temperature)

3. Lipaemic serum sample

4. Mislabeled samples

5. Old samples (stored beyond 7 Days)

6. Untested samples for TTI

3.4. Sample size determination

Using Fishers formula and a prevalence of 12.4 %, (Tigoi *et al.,* 2015) the study analyzed a total of 180 serum samples. This was upon considerations of equal distribution of the samples among the two regions of study being major transfusion centers. Below is the formula used to obtain the minimum sample size:

$$n = \frac{z^2 P(1-p)}{d^2}$$

n = minimum sample size

z = Statistic for level of confidence on normal distribution;

critical value set at 1.96 which corresponds to 95% confidence interval

p = residual rate of 12.4%

d = degree of precision set at plus or minus 5%

$$n = \frac{1.96^2 \times 0.124(1-0.124)}{0.05^2} = \mathbf{167}$$ Subjects

3.5. Sampling method and processing

All archived blood meeting the inclusion criteria were considered for testing. At both Nakuru and Nairobi RBTTCs, TTI whole blood samples were collected in the red top vacutainer and spun at a speed of 5000 revolutions per minute for 10 minutes for preparation of serum. Samples were initially separated based on gender, this was to ensure that the blood sample was gender representative. They were arranged randomly and a consecutive sampling technique applied (every second sample was picked for storage and analysis subsequently). This technique was applied until all the samples required for the study were obtained. The selected 180 whole blood samples were separated to form serum and aliquoted into 2ml vials and stored at -20oc. 100 microliters per plate was used for the test. Testing only commenced once all the serum samples had been obtained.

3.6. Laboratory testing for WNV IgG antibodies

An ELISA assay was used that targeted the immunoglobulin G in the sample. The West Nile Virus IgG Capture DxSelect™ ELISA kit that was used for analysis of the WNV is FDA approved and so further validations were not necessary. The principle of the test is based on the antibody capture technique. The expected result was either a positive or a negative. Briefly, a 100µL aliquot of the each serum sample (diluted 1:101 in Sample Diluent) was tested for the presence of WNV IgG antibodies. Two

96 well polystyrene plates were used. Each well was pre-coated with recombinant West Nile virus antigen. For each set of ELISA test run, two controls and a cut-off calibrator were included (refer to Appendix III).

3.6.1 Specificity of the Focus WNV IgG Assay used.

Assay Focus assessed specificity of the WNV IgG Assay by selecting fifteen different sera that were positive for IgG. The sera were treated with 5 μL of 1.43 M (10% v/v) 2-mercaptoethanol (2-ME). Treating with 2-ME caused 100% (15/15) of the samples to become IgG negative.

3.6.2 Reproducibility of the assay.

Reproducibility studies included Inter-lot Reproducibility, Inter/Intra-assay Reproducibility, and Inter-laboratory Reproducibility. In each assay, two sets of samples were masked duplicates. The kit's Inter-lot Reproducibility was assessed by testing five samples on three separate days with three separate technicians. For one lot, the samples were run in triplicate, and run in duplicate with the other two lots. Each of the three lots had a different lot of Antigen and Capture Wells. The assay's Inter/Intra-assay Reproducibility was assessed the by testing seven samples in triplicate, once a day, for three days. For detailed procedure of the assay, please refer to Appendix II.

3.7 Quality Assurance and Control

All the blood for TTI testing was stored in a cool box after collection for shipment. All the samples collected for the day were then shipped to the laboratory for testing.

First the donors' demographic data was checked against all samples for concurrence and recording. In cases where analysis for TTI was delayed and could not be done the same day, the samples were aliquoted into 2ml vials and stored at 2-8oC for analysis the following day. Only samples preserved at the right temperature were used in the study. The blood samples were spun to obtain serum. Reagents were stored at the correct temperatures to ensure that their integrity was maintained. Room temperatures were also monitored to ensure optimization of the reagents.

Commercial QC from DxSelect Company was used with two control levels. These were a Positive Control and a Negative Control. These were checked for validity before use. Every batch of samples analysed was accompanied by a positive and negative control. Results obtained for the samples were interpreted based on the validity of the control results obtained. The results were read by two people to ensure concurrence and uniformity. This was done independently and within the stipulated analytical time frame. Results from the two technologists were compared for concurrence and any discrepancies addressed immediately. Only concurrent results were used for analysis and those which were different were analysed by a third technologist as a tie-breaker in case they had to be included in the study.

3.8 Data management

OD readings at 450nm were generated following the ELISA assays (Table 4.3). The data was collected and stored in a hard cover book, Microsoft excel as well as SPSS software. Information stored in soft copies was protected from access by unauthorized persons by a password which was changed periodically. Data in hard

copies was stored in well secured lockable cabinets where only authorized persons could access them. All records were identified by study identification numbers. Records were stored and identified using unique laboratory numbers.

3.9 Data analysis

All data was analyzed using SPSS version 21. The results were presented in percentages using tables and charts as well as ranges, mean and standard deviation.

3.10. Ethical considerations/Approval

Ethical approval for conducting the study was obtained from the KNH/UON ethics review committee. Permission was also sought from the director in charge of KNBTS CENTRES. Donors' privacy and confidentiality was strictly observed. All procedures were performed in a standard manner to minimize harm and maximize benefit to study participants. Results were sent to the patients´ files in a timely manner to inform clinical decisions.

CHAPTER FOUR

RESULTS

4.1 Demographics of blood donors

Blood samples for 180 blood donors were analyzed. The donors had a mean age of 31.4 years (SD 7.6 years) ranging from 19 to 58 years. They were predominantly males constituting 143 in number (79.4%). About a half at 92 (51.1%) were sampled from Nairobi Regional Blood Transfusion Center (RBTC) while the rest 88 were sampled from Nakuru Regional Blood Transfusion Center (RBTC) (Table 4.1).

Table 4.1: Demographic characteristics of blood donors in Nakuru and Nairobi

Variables	Frequency (%)
Mean age (SD) years	31.4 (7.6)
Min-Max age years	19-58
Age categories	
19-35 years	138 (76.7)
36-45 years	32 (17.8)
46-55 years	9 (5.0)
>55 years	1 (0.6)
Gender	
Male	143 (79.4)
Female	37 (20.6)
Blood centers	
Nairobi	92 (51.1)
Nakuru	88 (44.4)

4.2 Blood group distribution

Majority (46.1%) of the blood samples were blood type O+ followed by B+ (20%) and 17.2% A+ (17.2%). The rest of the blood types contributed less than 10% each. In total, blood group O had more than half of the blood distribution of the sample, followed by blood group B (41%) and A (33%) (Table 4.2).

Table 4.2: Blood groups Types of samples tested

Variable	Frequency (%)
Blood group	
O+	83 (46.1)
O-	13 (7.2)
B+	36 (20.0)
B-	5 (2.8)
AB+	9 (5.0)
AB-	1 (0.6)
A+	31 (17.2)
A-	2 (1.1)

4.3 Seroprevalence of WNV IgG

WNV IgG prevalence in blood donors was at 15% with a 95% CI of between 10 and 20.5%. This is shown in Table 4.3

Table 4.3: ELISA 96 well plate results of the analyzed samples.

Software Version 3.02.1

Experiment File Path: C:\Users\Public\Documents\Experiments\FOCUS 2.xpt
Protocol File Path:
Plate Number Plate 2
Date #####
Time #####
Reader Type: ELx800
Reader Serial Numbe Unknown
Reading Type Reader

Procedure Details

Plate Type 96 WELL PLATE
Read Absorbance Endpoint
Full Plate
Wavelengths: 450
Read Speed: Normal

Results

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.05	0.05	0.076	0.072	0.082	1.726	0.061	0.136	0.056	0.171	0.074	0.078	450
B	0.935	0.067	0.082	0.095	0.1	0.09	0.058	0.072	0.085	0.069	0.17	0.088	450
C	0.492	0.082	0.095	0.221	0.148	0.07	0.063	0.107	0.054	0.066	0.079	0.111	450
D	0.459	0.092	0.116	0.062	0.167	0.052	0.069	0.135	0.418	0.097	0.098	0.096	450
E	0.074	0.09	0.053	0.19	0.06	0.052	0.07	0.11	0.074	0.089	0.333	0.102	450
F	0.094	0.118	0.059	0.072	0.139	0.074	0.334	0.069	0.181	0.088	0.085	0.294	450
G	0.097	0.11	0.126	0.135	0.055	0.064	0.108	0.096	0.135	0.106	0.101	0.169	450
H	0.105	0.087	0.075	0.067	0.103	0.098	0.125	0.092	0.155	0.087	0.116	0.045	450

A 0.05 0.05 0.076 0.072 0.082 1.726 0.061 0.136 0.056 0.171 0.074 0.078 450

B 0.935 0.067 0.082 0.095 0.1 0.09 0.058 0.072 0.085 0.069 0.17 0.088 450

C	0.492	0.082	0.095	0.221	0.148	0.07	0.063	0.107	0.054	0.066
	0.079	0.111	450							
D	0.459	0.092	0.116	0.062	0.167	0.052	0.069	0.135	0.418	0.097
	0.098	0.096	450							
E	0.074	0.09	0.053	0.19	0.06	0.052	0.07	0.11	0.074	0.089
	0.333	0.102	450							
F	0.094	0.118	0.059	0.072	0.139	0.074	0.334	0.069	0.181	0.088
	0.085	0.294	450							
G	0.097	0.11	0.126	0.135	0.055	0.064	0.108	0.096	0.135	0.106
	0.101	0.169	450							
H	0.105	0.087	0.075	0.067	0.103	0.098	0.125	0.092	0.155	0.087
	0.116	0.045	450							

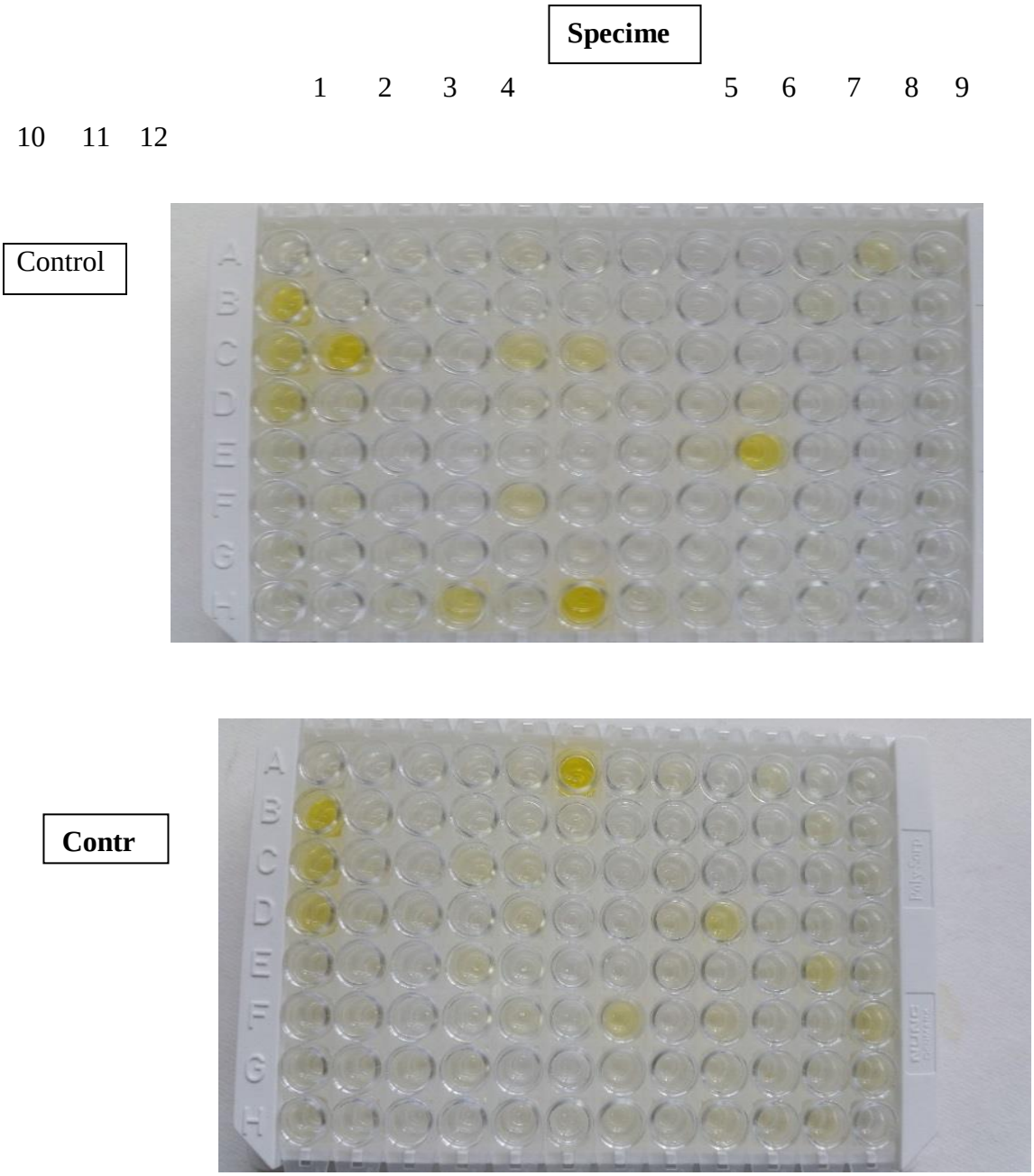

Figure 4.1: Test results of the ELISA 96 well after addition of the stop solution.

Table 4.4: Seroprevalence of West Nile Virus IgG

Variable	Frequency (%)	95% CI
WNV results		
Positive	27 (15.0)	10.0 - 20.5
Negative	153 (85.0)	79.5 - 90.0
Cross infection (WNV and TTI)		
Yes	15 (8.3)	4.4-12.2
No	165 (91.7)	87.8-95.6

The prevalence of cross infection of WNV and TTI was 8.3% (95% CI 4.4-12.2%)

4.4 Seroprevalence of West Nile Virus IgG by age, gender and blood center

Prevalence of WNV IgG was higher (16.7%) in donors in the age group between 19 and 35 years compared to those above 35 years (9.5%). However, the difference was not statistically significant (p=0.256). Similarly, though not statistically significant, females had a higher prevalence at 21.6% compared to males at 13.3%, p=0.206. Blood samples from the different centers did not show any difference in terms of IgG positivity with Nairobi having 14.1% of the blood donors positive, 16.3% in Nakuru (p=0.909) (Table 4.4).

Table 4.5: Seroprevalence of West Nile Virus IgG age, gender and blood center

	WNV		
	Positive (%)	Negative (%)	P value
Age			
19-35	23 (16.7)	115 (83.3)	0.256
>35	4 (9.5)	38 (90.5)	
Gender			
Male	19 (13.3)	124 (86.7)	0.206
Female	8 (21.6)	29 (78.4)	
Blood center			
Nairobi	13 (14.1)	79 (85.9)	
Nakuru	14 (16.3)	67 (83.8)	0.909

4.5 Testing for transfusion transmissible infections

Blood samples that were TTI positive made up 40% of the population. Those positive for other TTIs were mainly HIV (81.9%) while 12.5% were positive for Hepatitis B (Table 4.5).

Table 4.6: Other TTIs of different viruses

Other TTIs (n=72)	IgG WNV +ve	IgG WNV –ve	Frequency (%)
HIV	12 (16.7)	47 (65.2)	59 (81.9)
Hepatitis B	3 (4.2)	6 (8.3)	9 (12.5)
Hepatitis C	0 (1.4)	2 (1.4)	2 (2.8)
Syphilis	0	2 (2.8)	2 (2.8)
TTI Total	15	57	72 (40.0)
Negative TTI			108 (60.0)
TOTAL			180

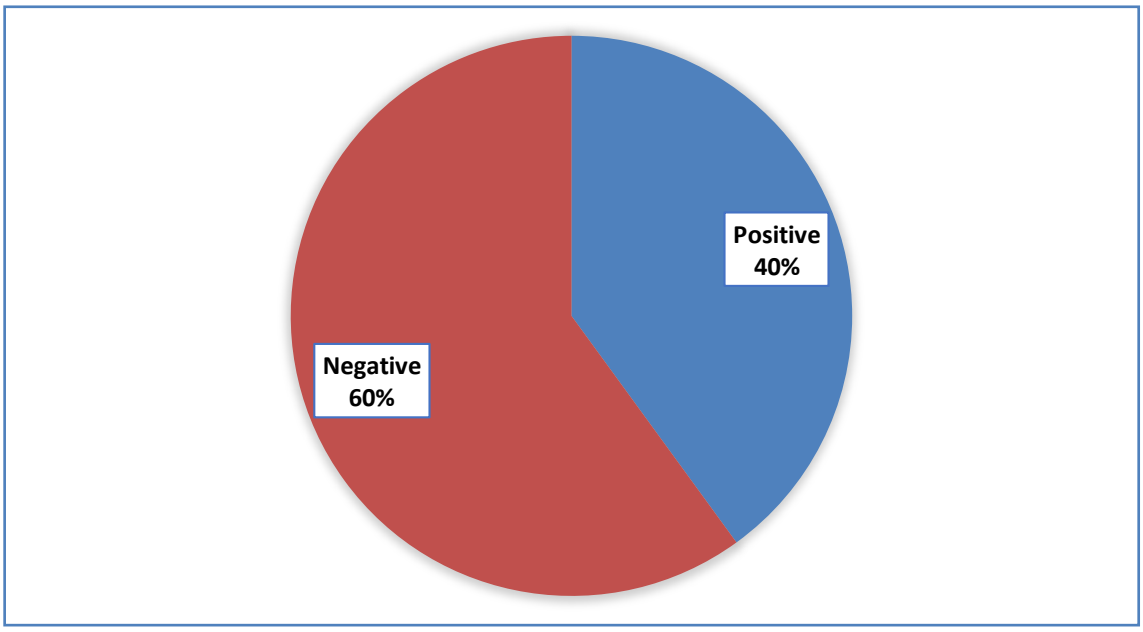

Figure 4.2: TTI results of the tested samples.

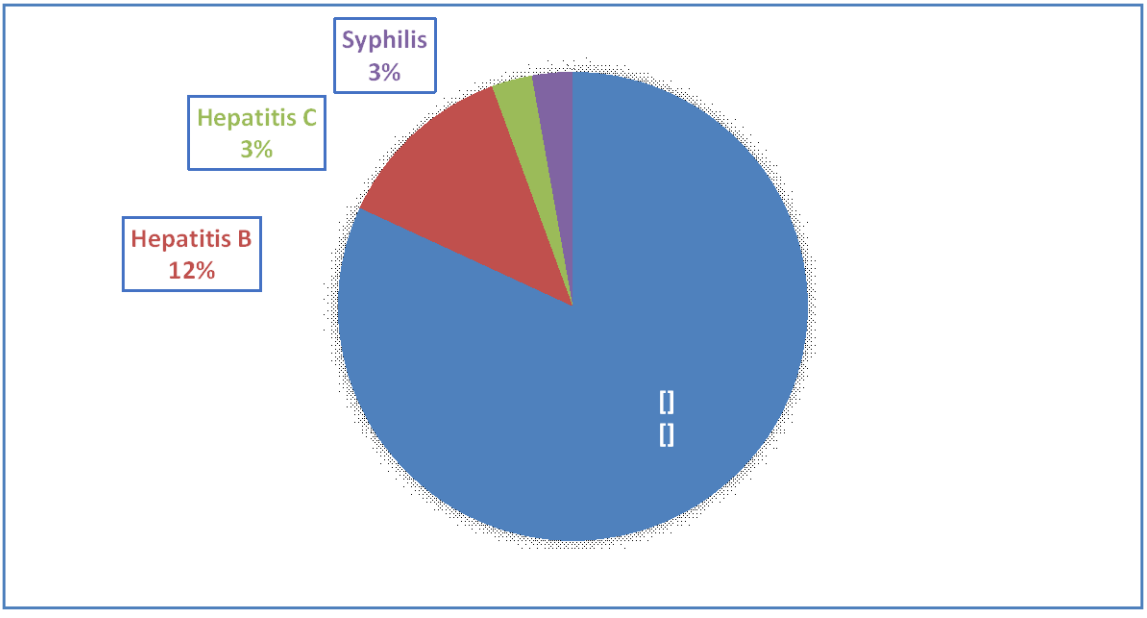

Figure 4.3: Other positive TTIs from the samples.

CHAPTER FIVE

DISCUSSION CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

A number of socio-demographic factors of blood donors were analyzed. In this study, a higher number of blood donors in the age group between 19 and 35 years were infected (16.7%) compared to those above 35 years (9.5%). This could be explained by the fact that WNV is a vector-borne disease that is transmitted by Culex mosquitoes and individuals in this age group may be more likely to be involved in outdoor activities hence exposure to mosquito bites (CDC, 2015; Pezzotti *et al*., 2011). This could explain the reason why a Ghanaian study reported most WNV infections occurring early in life (Wang *et al.*, 2009). It is also similar to data that shows more young people donate blood in low- and middle income countries (WHO, 2017). The blood samples from the different centers did not show any significant difference in IgG positivity. This shows that different cosmopolitan areas may have the similar WNV prevalence. In Kenya, most of the blood donors are school going children and older people do not donate regularly hence infections likely to be seen among the younger ages.

Despite males being the majority in this study, females had a higher prevalence (21.6%) of WNV IgG. This could be that women are more involved in outdoor activities such as washing laundry or near water bodies that put them at risk of infection. It was the opposite in a U.S. study where positive results for WNV were 63% higher among male than female donors (Betsem *et al.*, 2017). Another U.S.

report had a similar higher infection in males (Krow-Lucal *et al*., 2017). It could be that males in this population are more likely to be involved in activities or in areas where they are a higher risk of infection. Similar results were reported in a North Dakota study where the rate of infection was greatest for younger persons and men (Carson *et al.,* 2012). However, Krow-Lucal *et al.,* (2017) reported that the median age for WNV patients was 58 years probably because the older generations may be involved in outdoor leisure activities. The large proportion of males corresponds to the WHO data where globally 70% of blood donations are given by men (WHO, 2017). Though a US study showed that 53% of the donations were from men (Betsem *et al.,* 2017), they were still the majority. However, there was no significant association found with age and sex of the donor in this study similar to an Italian study (Pezzotti *et al.,* 2011). This could be because males and females of different ages are similarly exposed to the risk of WNV.

In this study, more than half of the samples were in blood group O. This is similar to what is reported that blood group O is the most common blood group (NHS, 2017).

WNV IgG positivity is an indication that an individual had previously been exposed to culex mosquitoes which were infected with the virus. Antibody M is the active Virus (In blood 3days- 30 days) while Antibody G is not the active virus but is in blood (2months to years).

This study showed a relatively high IgG seroprevalence among blood donors at 15%. Similar proportions have been reported in previous studies. Tigoi *et al* (2015) reported 12.4% prevalence in a population sampled in the lake basin areas of

Baringo, Naivasha and Tana River. However, a higher prevalence was reported in patients sampled in Western Kenya which showed 31% of them were positive for WNV (Muyeku *et al*, 2011). This is probably because it is an area prone to WNV infections. Other areas have reported lower prevalence such as a study in New Zealand that showed a lower prevalence of 2.1% in blood donors who had previously travelled to WNV endemic regions (Huang *et al.,* 2010). A study on Iranian donors reported that all were negative for WNV-specific antibody (Sharifi *et al.*, 2010).

In this study the IgG positivity from the two regions, Nairobi and Nakuru, did not show any differences. This could be because of the cosmopolitan nature of the study sites. Just like other studies this study has clearly shown that approximately 80% of people who are infected with WNV will not show any symptoms at all. 20% of people who develop illness have relatively mild disease with symptoms that include fever, headache, body aches, nausea and vomiting as well as swollen glands, skin rash on the chest, stomach and back. Symptoms may be experienced for a few days sometimes for weeks or even months (CDC, 2015). People with weakened immune function are at a higher risk for getting severely ill when infected with WNV. These symptoms may last several weeks to years and neurological effects can be permanent according to CDC. Less than 1% of those infected have severe symptoms such as meningitis and encephalitis. About seventy to eighty percent of those infected by the virus do not develop any symptoms.

However, a U.S. study showed that positive blood donations clustered according to WNV epidemic activity and the catchment areas of participating blood collection networks (Betsem *et al.,* 2017). This could also be the reason why the patients

sampled in Western Kenya had a higher WNV prevalence (Muyeku *et al*, 2011). The same was reported in a Turkey study where the WNV positive donors had risk factors such as outdoor activities and contact with mosquitoes (Ayturan *et al.,* 2011).

No study had previously been done on this IgG positivity. Further studies should be conducted to determine the causes of the high prevalence of WNV among TTI positive blood donors which leads to cross reactivity.

There is a risk of TTIs being transmitted from an infected donor through transfusion of blood (Zohreh *et al.,* 2010). In this study, WNV and other TTIs were identified. More than a third of the blood samples tested positive for TTIs. This puts those receiving blood donations at risk of getting infections (Mudassar *et al.,* 2014). The positive results were for HIV, hepatitis B and C and syphilis with HIV contributing the majority of the TTIs. Infections from these microorganisms can have serious repercussions on those receiving the blood donations as they are already ill. For example, HIV infection makes it harder for the body to fight off infections and diseases while hepatitis B can be chronic causing long term health problems (CDC, 2017).

5.2 Conclusion

The prevalence of WNV IgG was higher (16.7%) among those of ages 19 to 35. This is reflective of the fact that this is usually the common age group that donates blood.

The WNV IgG prevalence of 15% was relatively high. Therefore cosmopolitan areas may show a higher prevalence than non-endemic areas, but a much lower prevalence

than endemic areas. Even though these areas may not be endemic, it has been reported that those who travel to and from endemic areas can be infected with WNV.

Though the risk of TTIs has been greatly reduced by improvements in donor screening and testing, reports of newly identified infections or re-emerging infections such as West Nile virus cause ongoing challenges (Gharib et al., 2013). West Nile virus can be transmitted through transfusion with blood from an infected donor (Zohreh *et al.,* 2010) and because large volumes of blood or blood components are given to patients during transfusion therapy, the result is such that even a blood unit with low viral load may cause infection in the patient (Mudassar *et al.,* 2014).

This raises the need for blood transfusion services, for example in Nairobi and Nakuru, to develop contingency plans to ensure surveillance for emerging infections, assessment of their transmissibility by transfusion, actual likelihood of transmission and diseases associated with transmission (CDC, 2015). This is because the cosmopolitan nature of these areas results in some donors harboring WNV. It is therefore important to screen for WNV seeing that in low-income countries, up to 65% of transfusions are for children under the age of five years which contrasts with high-income countries where 76% of all transfusions are in patients over 65 years of age (WHO, 2017). There are also no medications to treat or vaccines to be used in the prevention of WNV (CDC, 2015).

5.3 Recommendations

i. Positive IgG samples should be investigated further for infectivity capability.

ii. KNBTS should develop a contingency plan to ensure surveillance for emerging infections including WNV

iii. Further studies may be required to determine whether there is a high prevalence in women but this study was limited to low number of blood donors

iv. Patients who display characteristics of WNV symptoms (coma, stiff neck, tremors, headache, vomiting, nausea) should be further investigated for WNV.

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APPENDICES

Appendix I: Data Collection Sheet

DATE OF SAMPLE COLLECTION: ……

Sample number …….

Location of collection……..

Date…….

Age……..

Gender…….

Region……..

Town…….
LAB REPORT

Routine TTI screening results

Positive…… Negative……(tick)
WNV RESULTS

1. WNV Positive…… 2.WNV Negative……

COMMENTS

Principal investigator's name……Sign……..

Technologist's name……. Sign…….

Date……

Appendix II: Procedure of Elisa Testing

ELISA PROTOCOL

TEST PROCEDURE

1. Bring all reagents to room temperature before use. Remove the Antigen Well packet from cold storage. To avoid condensation, allow micro-well strips to reach room temperature before opening the foil packet. If less than a full plate is to be used, return unused strips to the foil packet with desiccant and reseal completely. Store unused antigen wells at 2oC to 8oC. (Note: At the end of the assay, retain the frame for use with the remaining strips.)

2. Fill wells with 1X Wash Buffer solution (see Materials Supplied, above) and allow to soak for 5 minutes. Decant(or aspirate) the antigen wells and tap vigorously to remove wash buffer. Blot the emptied antigen Wells face down on clean paper towels or absorbent paper to remove residual wash Buffer.

3. Dispense 100 µL of the Sample Diluent into the "blank" wells and 100 µL of each diluted specimen, control or calibrator into the appropriate wells. (Note: For runs with more than 48 wells it is recommended that 250µL of each diluted sample first be added to a blank microtiter plate in the location corresponding to that in the ELISA wells. The samples can then be efficiently transferred into the Antigen Wells with a 100µL 8 or 12-channel pipettor.)

4. Cover plates with sealing tape (or place in a humid chamber), and incubate for 60 minutes at room temperature (20 to 25oC).

5. Remove sealing tape (or remove wells from the humid chamber), and empty the contents of the wells into a sink or a discard basin.

6. Fill each well with a gentle stream of 1X Wash Buffer solution from a wash bottle then empty contents into a sink or a discard basin.

7. Repeat wash (step 6) an additional 2 times.

8. Tap the antigen wells vigorously to remove 1X Wash Buffer. Blot the emptied Antigen Wells face down on clean paper towels or absorbent paper to remove residual 1X Wash Buffer.

9. Dispense 100µL conjugate to all wells, using a 100µL 8 or 12-channel pipettor.

10. Cover plates with sealing tape (or place in a humid chamber) and incubate for 30 minutes at room temperature (20 to 25oC).

11. Repeat wash steps 5 through 8.

12. Pipette 100µL of Substrate Reagent to all wells, using a 100µL 8 or 12-channel pipettor. Begin incubation timing with the addition of Substrate Reagent to the first well. (Note: Never pour the substrate reagent into the same trough as was used for the conjugate.)

13. Incubate for 10 minutes at room temperature (20 to 25oC).

14. Stop the reaction by adding 100µL of Stop Reagent to all wells using a 100µL 8 or 12-channel pipettor. Add the Stop Reagent in the same sequence and at the same pace as the Substrate was added. In antibody-positive wells, color should change from blue to yellow.

15. Gently blot the outside bottom of wells with a paper towel to remove droplets that may interfere with reading by the spectrophotometer. Do not rub with the paper towel as it may scratch the optical surface of the well. (Note: Large bubbles on the surface of the liquid may affect the OD readings.)

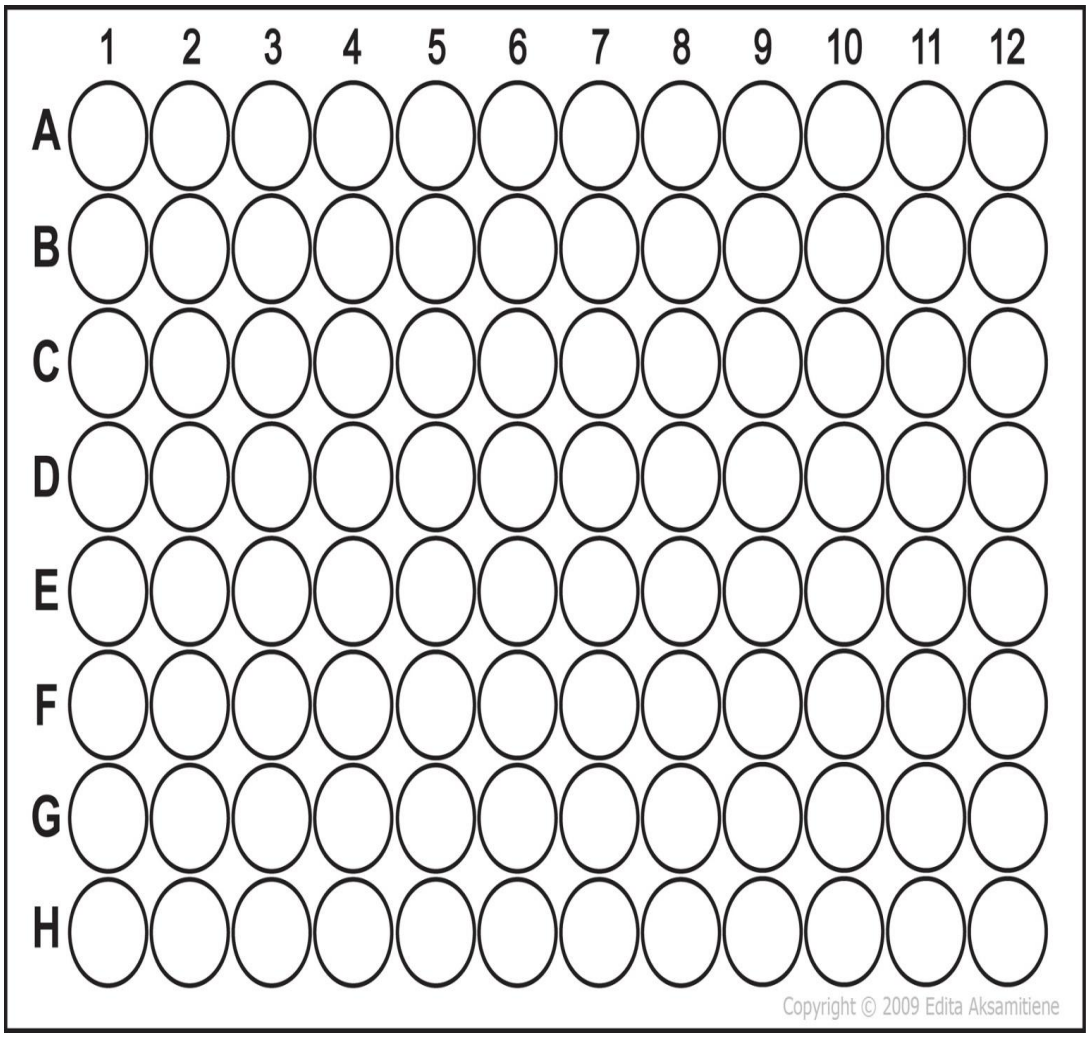
16. Measure the absorbance of each well within 1 hour of stopping the assay. Set the microwell spectrophotometer at a wavelength of 450 nm. Zero the instrument on the blank wells, or correct all ODs by manually subtracting the blank ODs.

CUT OFF

1. The Positive Control index values should be between 1.5 and 3.5.

The Negative Control index values should be less than 0.8

Appendix III: 96 Well Elisa Plate



Software Ver	3.02.1
Experiment F	C:\Users\NPHL\Desktop\christabel1.xpt
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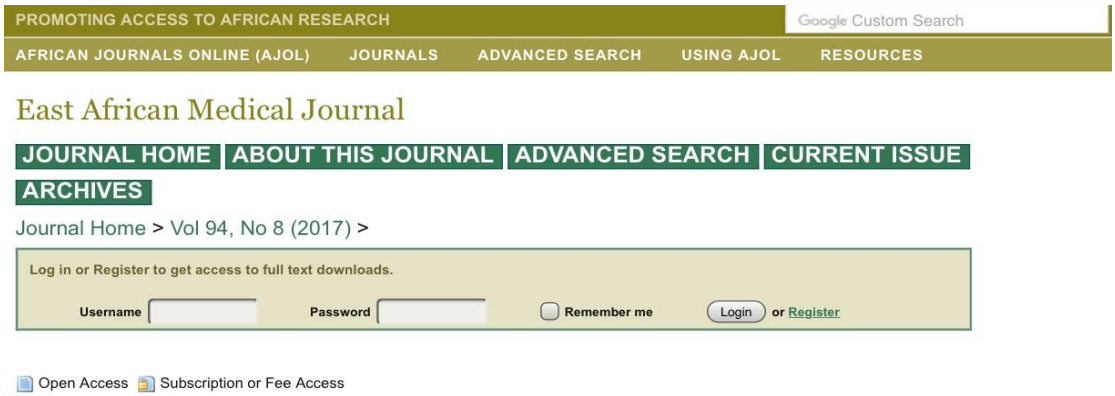
Date	7/19/2017
Time	1:10:42 PM
Reader Type:	ELx800
Reader Seria	Unknown
Reading Type	Reader
Procedure Details	
Plate Type	96 WELL PLATE
Read	Absorbance Endpoint
	Full Plate
	Wavelengths: 450
	Read Speed: Normal
Results	

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.056	0.094	0.135	0.105	0.216	0.099	0.071	0.088	0.069	0.145	0.391	0.134	450
B	1.075	0.069	0.125	0.101	0.079	0.091	0.126	0.101	0.079	0.225	0.088	0.082	450
C	0.609	1.678	0.073	0.127	0.511	0.498	0.111	0.093	0.078	0.111	0.122	0.115	450
D	0.622	0.131	0.115	0.14	0.171	0.17	0.112	0.129	0.261	0.109	0.086	0.093	450
E	0.17	0.072	0.088	0.058	0.092	0.102	0.098	0.232	1.312	0.092	0.091	0.104	450
F	0.093	0.271	0.076	0.089	0.416	0.119	0.12	0.071	0.122	0.118	0.075	0.119	450
G	0.203	0.17	0.175	0.078	0.098	0.104	0.112	0.12	0.107	0.172	0.114	0.048	450
H	0.159	0.095	0.206	0.741	0.116	2.041	0.148	0.199	0.091	0.155	0.1	0.043	450

Appendix IV: Published Article



IGG-antibody seroprevalence of West Nile Virus among blood donors in Nairobi and Nakuru regional blood transfusion testing centers in Kenya

C.J. Soi, S Kibet, A Mbugua, P Maturi, L Hiyan

Abstract

Background: West Nile Virus (WNV) is an arbovirus transmitted by infected mosquitoes which cause most of its incidence (CDC, 2015). It is transmitted by the culex mosquito which is prevalent in Kenya.

Objective: To determine and compare the sero prevalence of WNV among blood donors in Nairobi and Nakuru Regional blood transfusion testing centers in Kenya.

Study design: A cross-sectional study

Setting: It was carried out in two Regional Blood Transfusion Centers (RBTCs) which are based in Nairobi and Nakuru. These two centers are associated with possible low and high prevalence respectively.

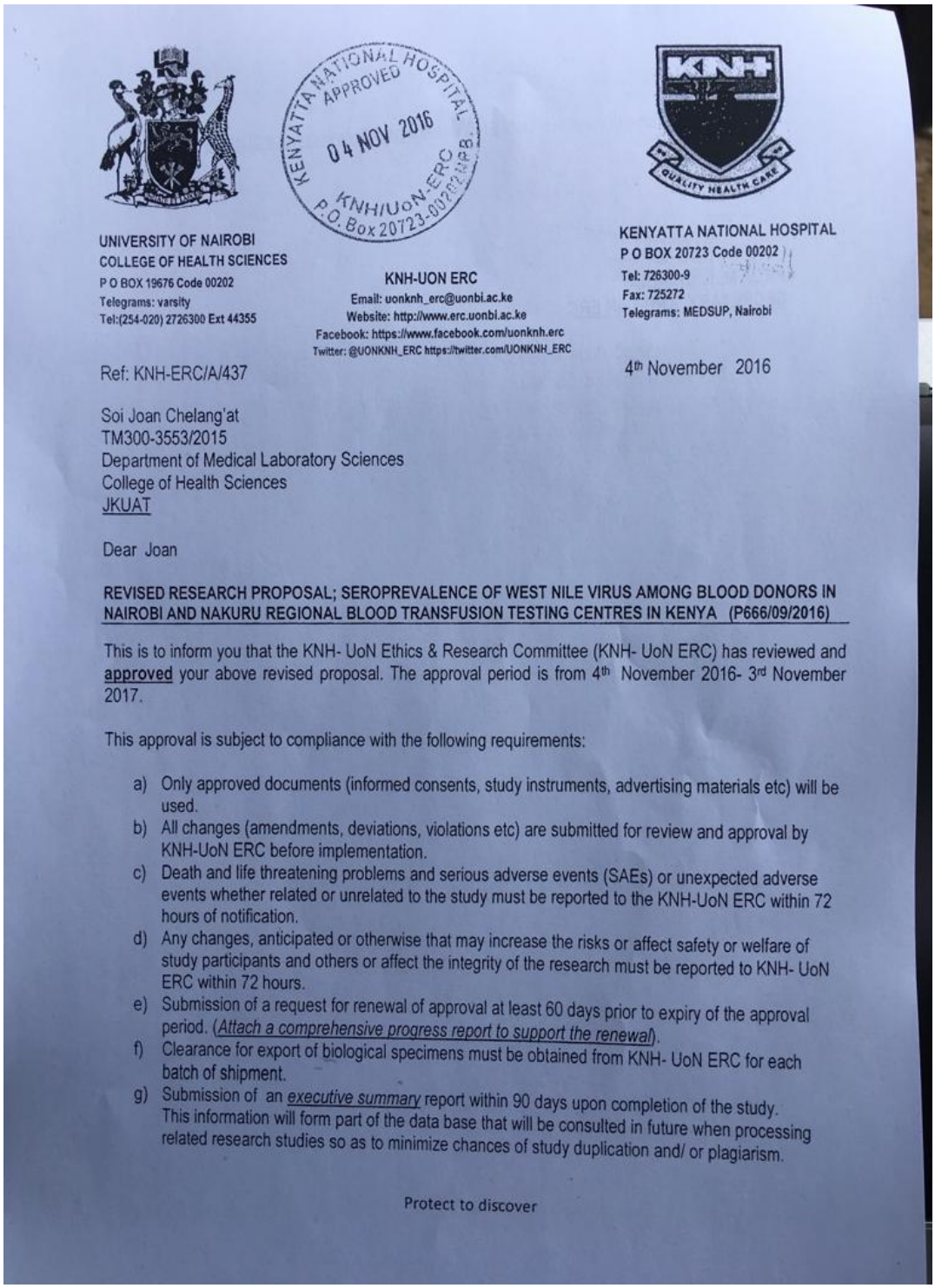
Subject: A total of 180 blood samples were randomly selected over a period of one month. These blood samples were tested for WNV IgG using ELISA. Results: Majority of the donors were below 35 years of age and were predominantly male. WNV IgG prevalence was 15% in blood donors (95% CI 10-20.5%). Prevalence of cross infection of TTI and WNV was 8.3% (95% CI 4.4- 12.2%). The prevalence of WVN IgG was highest in the 19-35 years' age group (16.5%) and females (21.6%) though the results were not statistically significant. There was no difference in the IgG positivity between the different centers.

Conclusion: Infection with WNV should be of public health concern because about a fifth of those infected with WNV develop illness. About 10% of those who develop neurological symptoms succumb to the disease.

Full Text:

EMAIL FULL TEXT
DOWNLOAD FULL TEXT

Appendix V: KNH/UON ERC Approval



KENYATTA NATIONAL HOSPITAL APPROVED 04 NOV 2016 KNH/UoN-ERC P.O. Box 20723-00202 Nairobi

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Ref: KNH-ERC/A/437

4th November 2016

Soi Joan Chelang'at
TM300-3553/2015
Department of Medical Laboratory Sciences
College of Health Sciences
JKUAT

Dear Joan

REVISED RESEARCH PROPOSAL; SEROPREVALENCE OF WEST NILE VIRUS AMONG BLOOD DONORS IN NAIROBI AND NAKURU REGIONAL BLOOD TRANSFUSION TESTING CENTRES IN KENYA (P666/09/2016)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above revised proposal. The approval period is from 4th November 2016- 3rd November 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.

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

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 Chairperson, KNH- UoN ERC
The Assistant Director, Health Information, KNH
Supervisors: Dr.Peter Maturi(UON), Dr.Amos Mbugua(JKUAT)