SEROPREVALENCE OF DENGUE FEVER VIRUS IN THE ADULT KENYAN POPULATION IN NAIROBI, ELDORET AND KISUMU REGIONS

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DECLARATION

I hereby declare that this is my original work and has not been presented for the award of a degree in any other university.

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DEDICATION

This works dedicated to my husband Alex and children, Aaron and Angel for their support throughout the study period. You are God's precious gift to me.

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I am most grateful to the Almighty God for the strength He gave me to carry out this research and write the findings.

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Finally I am grateful to my classmates, KACP Journal club and lecturers for their role in critiquing my research proposal.

LIST OF ABBREVIATIONS

AIDS - Acquired Immune Deficiency Syndrome

ADE - Antibody Dependent Enhancement

DENV- Dengue Virus

DF - Dengue Fever

DHF - Dengue Hemorrhagic Fever

DNA – Deoxyribonucleic acid

DSS - Dengue Shock Syndrome

NSI – Nonstructural protein1

ELISA - Enzyme Linked Immunosorbent Assay

HIV- Human Immunodeficiency Virus

IgG - Immunoglobulin G

IgM - Immunoglobulin M

KNH- Kenyatta National Hospital

KAVI- Kenya Aids Vaccine Initiative

UON- University of Nairobi

ERC - Ethical Review Committee

RBTCs- Regional Blood Transfusion Centres

PCR - Polymerase Chain Reaction

UNITID - University of Nairobi Institute of Tropical and Infectious Diseases

DDSR-Division of Disease Surveillance and Response

EAPHLN- East Africa Public Health Laboratory Network

KNBTs- Kenya National Blood Transfusion services

NML- National Microbiology Laboratory

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ABSTRACT

Background: - Dengue virus (DENV) is a mosquito-borne pathogen causing Dengue fever (DF) and dengue haemorrhagic fever (DHF). It is spread by the *Aedes* mosquitoes which are container breeders and live in close proximity to humans. This is an important factor as these mosquitoes therefore spread this disease with ease especially in the populated urban areas. DENV is the most rapidly spreading mosquito borne viral disease in the world causing an estimated 50–100 million DENV infections and hundreds of thousands of cases of DHF/DSS annually, with children bearing much of the disease burden.

Broad objectives: - To determine Dengue fever hotspots in selected regions in Kenya.

Study method

This was a cross-sectional descriptive study. Permission to do the study was sought from KNH-UoN Ethical review committee and Kenya National Blood Transfusion Services after which blood samples were collected from consenting adults donating blood at the regional blood transfusion centres in Nairobi, Eldoret and Kisumu. Plasma was then screened for anti-dengue virus IgG antibodies using Dengue DxSelect™ IgG in-house ELISA, giving the prevalence. The data was analysed using SPSS.

Results: - A total of 490 study participants were randomly sampled during the blood donation exercise in Kisumu, Eldoret and Nairobi: 61 (16%) were female and 326 (82%) were male. They then underwent a standardized interview and were blood sampled. The median age was 25 years with the youngest participant being 18 and the oldest 64 years. 387 plasma samples were tested for anti-dengue IgG antibodies. From Nairobi region, 99 samples were screened for anti-dengue IgG giving a prevalence of 11.1% (11/99). From Eldoret a total of 138 samples were screened giving a prevalence of 2.2% (3/138). In Kisumu, 150 samples were screened giving a prevalence of 5.4% (8/150). The presence of dengue fever virus antibodies in the adult Kenyan population

was found to be at 5.7% (22/387).

Conclusion: - The prevalence of dengue in the adult Kenyan population in Nairobi was 11.1%, Kisumu 5.4% and Eldoret 2.2%. This therefore means that there is exposure to dengue virus and hence possibility of outbreaks in these regions where no outbreaks have ever been reported. In addition there seems to be a marked increase of dengue activity in Kisumu since the last study done in 2011 showed dengue seroprevalence was at 1.1%. Improved diagnosis and surveillance of dengue fever virus would help predict future outbreaks.

1.0 INTRODUCTION

Dengue fever is a viral infection that is fast gaining importance at a global level (Jacobs, 2000). Most of the world's population is at risk. There are four dengue virus serotypes DENV-1, DENV-2, DENV-3 and DENV-4 (De Paula and Fonseca, 2004). East Africa has three main serotypes DENV-1, 2 and 3 (WHO, 2009). The emergence and spread of all four dengue viruses ("serotypes") from Asia to the Americas, Africa and the Eastern Mediterranean regions represents a global pandemic threat (WHO, 2012, Gubler and Clark, 1995). Although the full global burden of the disease is still uncertain, the patterns are alarming for both human health and the economy.

Dengue fever is spread by the *Aedes* mosquito which prefers the tropical climate (a zone in a range of latitudes between 23.5^o South to 23.5^o North; temperatures remains high all over the year). Its presence is also determined by temperature, humidity and rainfall patterns. However, changing global climatic patterns have seen the mosquito permeate virgin areas (Halstead, 1992). These emerging areas include areas like the Americas. This vector is mainly found around human habitation especially in urban areas. Human activities are also known to influence the mosquito's occurrence. Marked migration has led to the mushrooming of urban housing that has poor sanitation and deplorable sewage disposal all of which serve as suitable breeding sites for the mosquitoes.

This disease has non-specific presentation leading to marked misdiagnosis (Amexo et al., 2004)) It presents with a biphasic fever. Other presentations include joint pain, headache and generalized muscle pain. In severe cases, the patients present with bleeding complications that can prove fatal. A small percentage of the patients with Dengue fever progress to the severe form of disease, Dengue Hemorrhagic Fever (WHO, 2009). Surveillance and diagnosis of this disease has also proved difficult. This is due to poor diagnosis and the non-specific clinical presentation.

Clinical management is wanting with lots of room for improvement. Diagnosis has markedly improved with the introduction of dengue specific diagnostic tools. However marked cross reactivity of antibodies within the Flavivirus family has been a major setback (Calisher et al., 1989). This serves to give misleading figures on the incidence and prevalence of the disease. These factors serve to confirm the importance of mapping out emerging and re-emerging areas of infection.

The transmission of infectious and contagious agents through the transfusion of blood derivatives and components is characterized by delayed adverse reactions that increase the risks to recipients. Transfusion-transmitted dengue poses a risk to transfusion safety (Faddy et al., 2013). However, serological testing of blood donors does not guarantee total safety from possible transmission of infectious and contagious agents. DENV has been detected in donated blood from asymptomatic volunteers (Tezcan et al., 2014)

The development of a suitable vaccine is expected to be the mainstay of disease control.

2.0 LITERATURE REVIEW

Dengue virus (DENV) is a member of the *Flavivirus* genus of the *Flaviviridae* family, which includes several viruses that are threats to public health, including yellow fever virus (YFV), West Nile virus (WNV), Japanese encephalitis virus (JEV), and tick-borne encephalitis virus (TBEV) (Burke DS, 2001). There are four serotypes (termed DENV-1 to DENV-4) and at least three genetic groups (genotypes) within each serotype. Each of the four serotypes of DENV is capable of causing the full spectrum of clinical manifestations following infection, including asymptomatic infection, dengue fever (DF), and the most severe disease, dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). DENV causes significantly more human disease than any other arbovirus, as evidenced by the estimated 50–100 million DENV infections and hundreds of thousands of cases of DHF/DSS occurring annually; with children bearing much of the disease burden (WHO, 2009, Gubler and Meltzer, 1999). During the past five decades, the incidence of dengue has increased 30-fold (Fig. 1).

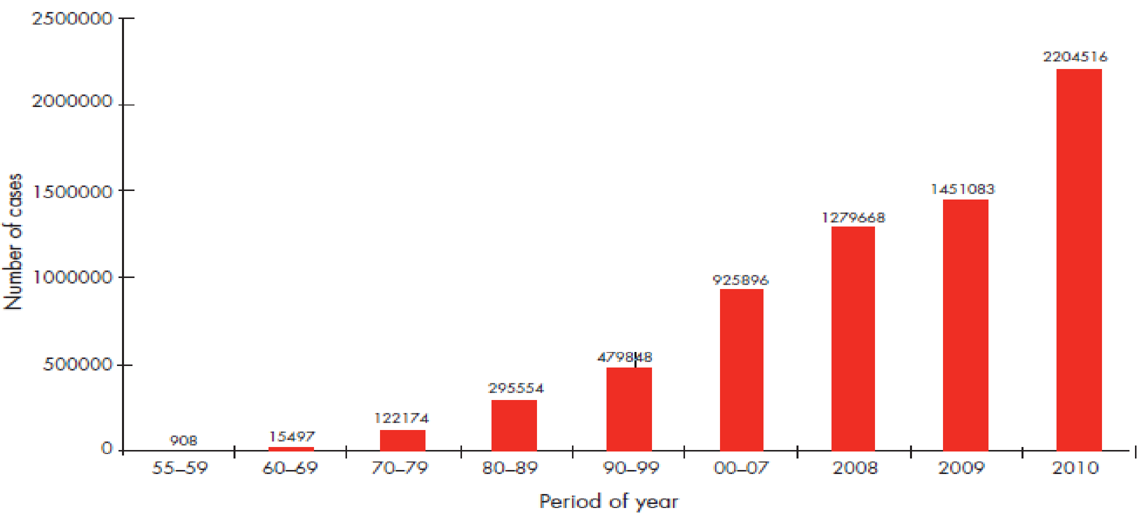

Figure 1. Average number of dengue and severe dengue cases reported to WHO annually in 1955-2007 and number of cases reported recently 2008-2010.

Source: WHO, 2012

Some 50–100 million new infections are estimated to occur annually in more than 100 endemic countries (WHO, 2012, Halstead, 1999) (Fig. 2) with a documented further spread to previously unaffected areas (CDC, 2010, La Ruche et al., 2010) (Fig. 3.) every year hundreds of thousands of severe cases arise, including 20 000 deaths (Gubler and Meltzer, 1999) 264 disability-adjusted life years per million population per year are lost (Cattand et al., 2006) at an estimated cost for ambulatory and hospitalized cases of US$ 514–1394 (Suaya et al., 2009)often affecting very poor populations. The true numbers are probably far worse, since severe underreporting and misclassification of dengue cases have been documented (Suaya et al., 2007(Beatty et al., 2011).

Dengue disease imposes a substantial economic liability (Suaya et al., 2009), and DHF/DSS remains a leading cause of hospitalization and death of children in at least eight Southeast Asian countries (WHO, 2009). Compared with other diseases and their respective burdens, dengue can cause as much or greater human suffering than other communicable diseases in some of the most affected regions. In Latin America and the Caribbean, for example, by the 1990s dengue was causing a similar burden of disease as meningitis, hepatitis, malaria, the childhood cluster of diseases (polio, measles, pertussis, diphtheria and tetanus) or tuberculosis (Meltzer et al., 1998).

For Africa, there are insufficient data from endemic countries to make even rough estimates of the disease burden. In a recent publication, 22 countries in Africa have reported sporadic cases or outbreaks of dengue from 1960-2010 (Amarasinghe et al., 2011).

The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines or maps represent approximate border lines for which there may not yest be fill agreement.

Data Source: World Health Organization Map Production: Public Health Information and Geographic Information Systems (GIS) World Health Organization

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Countries at Risk of Dengue, 2008

Figure 2. World map showing the areas at risk of dengue virus as of 2008

Source: WHO, 2008

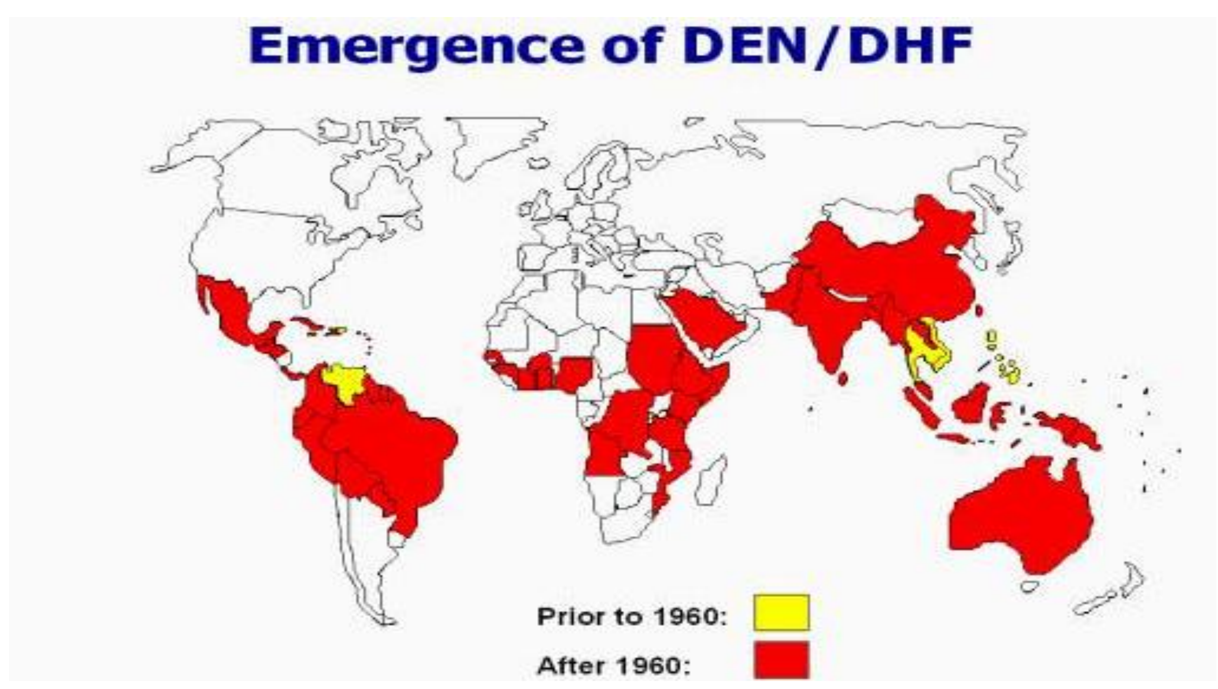


Figure 3. **Geographic spread of dengue*****. The countries in yellow in South America and Far East were the only places with evidence of dengue cases prior to 1960. After 1960, dengue had spread geographically (red).***

Source: World Health Organization

For eastern Africa, the available evidence so far indicates that DENV-1, -2 and -3 appear to be common causes of acute fever. Examples of this are outbreaks in the Comoros in various years (1948, 1984 and 1993, DENV-1 and -2) (Boisier et al., 1994) and Mozambique (1984 to 1985, DENV-3) (Gubler et al., 1986) (Table 1). While dengue may not appear to be a major public health problem in Africa compared to the widespread incidence of malaria and HIV/AIDS, the increasing frequency and severity of dengue epidemics worldwide calls for a better understanding of the epidemiology of dengue infections with regard to the susceptibility of African populations to dengue and the interference between dengue and the other major communicable diseases of the continent.

Dengue fever is spread by the *Aedes* mosquito which prefers the tropical climate (latitudes 23.5^o S to 23.5^o N). Its presence is also determined by temperature, humidity and rainfall patterns. However, changing global climatic patterns have seen the mosquito permeate virgin areas like the Americas (Halstead, 1992). Co-circulation of multiple serotypes is common within the countries that are most affected by DENV (Mackenzie et al., 2004). The rapid and intense spread of DENV to most of the tropical and subtropical world has resulted from spread of the vector, introduction of multiple dengue serotypes, and greatly increased urbanization and movement of people into and out of endemic regions (Kyle and Harris, 2008, Ooi and Gubler, 2009, Wilder-Smith and Gubler, 2008). This has led to the classification of dengue as an emerging infectious disease and has resulted in intensified efforts to develop an effective vaccine.

The most advanced vaccine candidate, which is based on live-attenuated chimeric yellow fever-dengue virus, has progressed to phase III clinical trials (Guy et al., 2011). Several other live-attenuated vaccines, as well as a subunit and a DNA vaccine, are in early stages of clinical development (Coller and Clements, 2011). Additional technological approaches, such as virus-

vectored and inactivated vaccines, are under evaluation in preclinical studies (Schmitz et al., 2011). Challenges to vaccine development include the need to provide protection against all four dengue viruses, as well as resolving questions about the immune correlates of protection. While proof of concept of vaccine efficacy is currently still missing, successful progression of ongoing efficacy trials could lead to the availability of a vaccine in 2–4 years.

It has been noted that several of the arboviral infections are actually expanding geographically as noted by the spread of dengue and re-emergence of yellow fever virus in South America (Riaz et al., 2009). Both yellow fever and dengue are transmitted between humans by *Aedes aegypti* which are anthropophilic mosquitoes that breed in urban dwellings. Dengue however appears to be more widespread than the yellow fever virus. It may be that dengue occurs in urban areas whereas yellow fever occurs in remote settings.

In 2006, an outbreak of dengue fever virus occurred in Karachi. A cross-sectional study was done in a tertiary hospital from January to December. A positive IgM titre was then assessed. An estimated 65.7% of the patients who were suspected to have had dengue had positive IgM titres (Riaz et al., 2009). The study showed a rise in the endemicity of the disease as noted by the results obtained. An epidemiological study done in 2010 at Kassala, Eastern Sudan during the period of August through to November had an 81% confirmed dengue fever infection. This was confirmed using ELISA IgM serology (Abdallah et al., 2012). In Kenya, a study in Western Kenya was done to assess the seroincidence and seroprevalence of dengue. Banked sera was obtained and analyzed for antibodies to dengue virus using an IgG indirect ELISA. The seroprevalence was found to be at 1.1% and an incidence of 8.5 seroconversions per 1000 persons per year in this study population (Blaylock et al., 2011). A study to review the trends and outbreaks of arboviruses in Kenya over the last ten years was done in 2001.The study reviewed

classifications, incidence, outbreak activity in Kenya, mode of transmission, recognition of cases, management and control. It showed an increased frequency of outbreaks and detection of arbovirus activity in humans and vectors in the last ten years (Sang and Dunster, 2001). The Division of Disease Surveillance and Response (DDSR) in April, 2013 reported confirmed DENV outbreaks in Mombasa Kenya. Of 148 blood samples collected and tested in Healthcare facilities using RT-PCR 56% were positive for DENV. The identified DENV types include DENV-1 (69 per cent), DENV-2 (28 per cent), and DENV-3 (3 per cent); at least one sample was positive for two DENV serotypes (DENV-1 and 2). This is the second town in Kenya to have reported cases of dengue since 2011; cases were reported in Mandera town in North Eastern Kenya in September 2011 (1300 cases) and again in January 2013 (190 cases). The importance of recognition of cases and their diagnosis is critical in management and control.

2.1 Epidemiology

Reported incidence rates of dengue have increased worldwide in the recent decades. Dengue was reported in Africa in the late 19th and early 20th centuries. Epidemics were reported in Zanzibar (1823, 1870), Burkina Faso (1925), Egypt (1887, 1927), South Africa (1926–1927), and Senegal (1927–1928) (Gubler and Clark, 1995, WHO, 2009) (Table 1). Although dengue exists in the WHO African Region, surveillance data are scanty. Outbreak reports exist, although they are not complete, and there is evidence that dengue outbreaks are increasing in size and frequency (Nathan MB, 2007)**.** The reasons for this dramatic global emergence of dengue as a major public health problem are complex and not clearly understood. This is mainly due to the under recognition and under reporting in Africa because of the low awareness by health care providers, other prevalent febrile illnesses and the lack of diagnostic tests and systematic surveillance

(Amarasinghe et al., 2011). Africa has a wide distribution of the *Aedes* which acts to increase the spread of Dengue fever virus.

The first reported epidemics of dengue fever occurred in 1779 to 1780 in Asia, Africa and North America (Gubler and Clark, 1995). The three outbreaks that occurred almost simultaneously indicated that the viruses and the mosquito vectors had been distributed worldwide for almost 200 years (Gubler and Clark, 1995). Reported epidemics that followed included those in Zanzibar, Burkina Faso, Egypt, South Africa, Senegal, Sychelles, Re-union islands, Comoros and most from the Eastern Africa region.

Table 1:- Countries with Evidence of dengue virus transmission in Africa

Locally acquired, n=7

Country	Year	Serotype
Egypt	1779, 1887, 1927	Unknown
Eritrea	2005	Unknown
Cape Verde	2009+	3
Mauritius	2009	Unknown
Ré-union Islands	1977–1978†	2
Seychelles	1977–1979†	2
Sudan	1984-1986	1 and 2
Locally and travel acquired, n=15		
Angola	1986, 1999-2000 ‡	Unknown
Burkina Faso	1925 1983-1986 2003–2004,§ 2007‡	Unknown 2 Unknown
Cameroon	1987–1993,§ 1999–2002,‡ 2000–2003,§ 2006‡	Unknown
Comoros	1943–1948 1984, 1992–1993†	Unknown 1 and 2
Djibouti	1991–1992†	2
Côte d'Ivoire	1982	2
	1998	1
	1999–2002‡	Unknown
	2008	3
Ghana	1932, 1987–1993§ 2002–2005	Unknown 2
Kenya	1982 1984–1986	2 1 and 2

Madagascar	1943–1948 2006	Unknown 1
Mozambique	1984–1985†	3
Nigeria	1964–1968	1
Senegal	1979	1
	1980–1985	2 and 4
	1990, 1999	2
	2007‡	Unknown
	2009	3
Somalia	1982, 1985–1987 1992-1993	2 2 and 3
South Africa	1927†	1
Zanzibar	1823, 1870, 2010‡	Unknown

Travel/expatriate, n=12

Benin	1987–1993§	Unknown
DRC	1999–2001,‡ 2007§	Unknown
Ethiopia	1999–2002,‡ 2007§	Unknown
Equatorial Guinea	1999–2002‡	Unknown
Gabon	1999–2002‡	Unknown
Mali	2008	2
Namibia	1999–2002,‡ 2006‡	Unknown
Rwanda	1987–1993§	Unknown
Tanzania	1987–1993,§ 1999–2002,‡ 2006,‡ 2010‡	Unknown
Togo	1987–1993,§ 1999–2002‡	Unknown
Uganda	1999–2002‡	Unknown
Zambia	1987–1993§	Unknown

†Large local outbreaks.

‡TropNet Europ Network and ProMED.

§Seroprevalence study.

Emergence of dengue fever (DF) and dengue hemorrhagic fever (DHF) has also been dramatic in the Americas region. In an effort to prevent urban yellow fever that is also transmitted by *Ae. aegypti*, the Pan American Health Organization organised a campaign that eradicated *Ae. aegypti* from most Central and South American countries in 1950-1960s (Gubler and Clark, 1995). But, the dramatic increase in the incidence of disease caused by DENV in the Americas over the past

three decades is due in large part to the geographic expansion of *A. aegypti* after the decline of vector-control efforts (Gubler, 1998).

Dengue virus currently has no vaccine that can control the infection. Disease prevention and mosquito control through community efforts should aim to reduce larval breeding sources. Improved, proactive, laboratory based surveillance systems can provide guidance and warn on impending epidemics. Surveillance will serve to alert the general public and physicians on the importance of the disease and lead to proper diagnosis and proper treatment of the infections.

2.2 Dengue outbreaks in Kenya

The Division of Disease Surveillance and Response (DDSR) in the Ministry of Health reported an outbreak of dengue fever in Mombasa County in Coastal region of Kenya in March 2013. Mombasa County last reported dengue fever outbreak in 1982 (DDSR, 2013) although cases were reported in the neighbouring North Eastern province, in Mandera East district in 2011 and 2013 affecting approximately 1300 and 190 people respectively (DDSR, 2013). Although the first laboratory confirmed case was reported by Kenya Medical Research Institute (KEMRI) on 7th March 2013, a retrospective review of hospital records indicated that the first case was confirmed in a private laboratory on 7th January 2013. From this review, there were at least 3 cases in January and 2 cases in February that had been confirmed in private laboratories and this could be linked to the outbreak in Mandera East in January. The serotype that was found in Mandera was mainly type 3 while the serotype in Mombasa was serotype 1. Therefore, based on the different serotypes, it is possible that these were two independent outbreaks as opposed to importation of the disease from Mandera into Mombasa. Between 7th March and 26th April 2013, 83 of 148 (56%) blood samples collected from residents of Mombasa who visited health facilities with dengue-like illness tested positive for dengue virus (DENV) infection by PCR diagnostic technology (EAPHLN-K, 2013).

2.3 Transmission of Dengue

2.3.1 The virus

Dengue virus (DENV) is a small single-stranded RNA virus comprising four distinct serotypes (DENV-1 to -4). These closely related serotypes of the dengue virus belong to the genus *Flavivirus*, family Flaviviridae. The mature particle of the dengue virus is spherical with a diameter of 50nm containing multiple copies of the three structural proteins, a host-derived membrane bilayer and a single copy of a positive-sense, single-stranded RNA genome (Cleaves and Dubin, 1979). The genome is cleaved by host and viral proteases into three structural proteins (capsid, C, the precursor of membrane, prM, protein, M and envelope, E) and seven nonstructural proteins (NS) (Bartenschlager and Miller, 2008) (Murray et al., 2008)

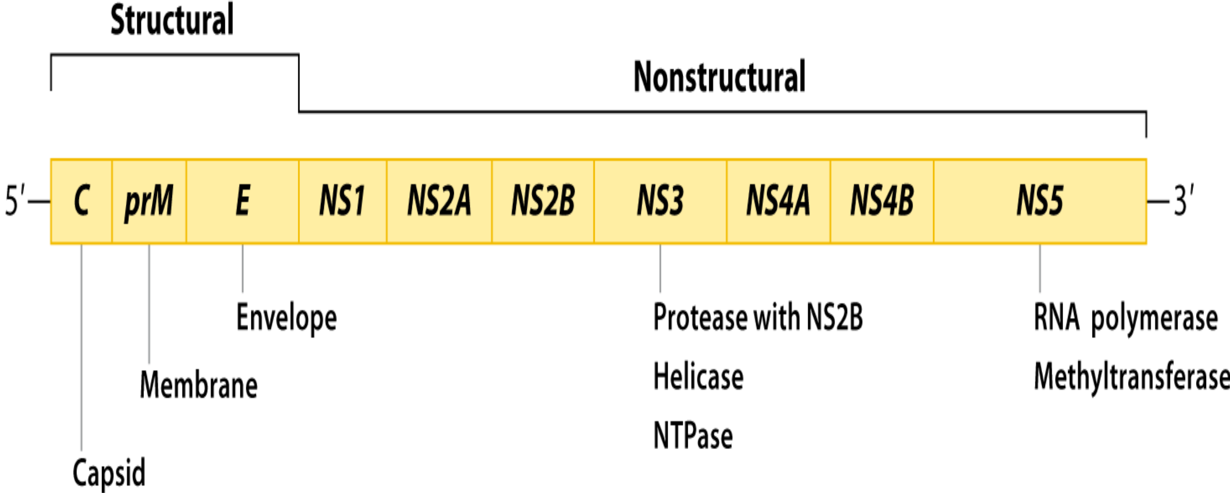


Figure 4. Dengue Virus genome structure showing the three structural proteins C, prM and E and seven non-structural proteins NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. (Murphy BR, Whitehead SS. 2011 Ann. Rev. Immunology 29:587-619)

Distinct genotypes or lineages (viruses highly related in nucleotide sequence) have been identified within each serotype, highlighting the extensive genetic variability of the dengue serotypes. Purifying selection appears to be a dominant theme in dengue viral evolution, however, such that only viruses that are “fit” for both human and vector are maintained. Among them, “Asian” genotypes of DENV-2 and DENV-3 are frequently associated with severe disease accompanying secondary dengue infections (Leitmeyer et al., 1999) (Lanciotti et al., 1994, Messer et al., 2003). Intra-host viral diversity (quasispecies) has also been described in human hosts.

2.3.2 The vectors

The various serotypes of the dengue virus are transmitted to humans through the bites of infected *Aedes* mosquitoes, principally *Ae. aegypti* (Rhodain F, 1997). This mosquito is a tropical and subtropical species widely distributed around the world, mostly between latitudes 35 ^{0}N and 35 ^{0}S. These geographical limits correspond approximately to a winter isotherm of 10 ^{0}C. *Ae. aegypti* has been found as far north as 45 ^{0}N, but such invasions have occurred during warmer months and the mosquitoes have not survived the winters. Also, because of lower temperatures, *Ae. aegypti* is relatively uncommon above 1000 metres.

Dengue viruses mainly circulate between human and vector mosquitoes and the vector presence is a limiting factor of transmission (Higa, 2011). The immature stages are found in water-filled habitats, mostly in artificial containers closely associated with human dwellings and often indoors. Studies suggest that most female *Ae. aegypti* may spend their lifetime in or around the houses where they emerge as adults. This means that people, rather than mosquitoes, rapidly move the virus within and between communities. Dengue outbreaks have also been attributed to *Aedes albopictus* (Effler et al., 2005), *Aedes polynesiensis* and several species of the *Aedes scutellaris* complex (WHO, 2009). Each of these species has a particular ecology, behaviour and

geographical distribution. In recent decades *Aedes albopictus* has spread from Asia to Africa, the Americas and Europe, notably aided by the international trade in used tyres in which eggs are deposited when they contain rainwater (Knudsen, 1995). The eggs can remain viable for many months in the absence of water (Knudsen, 1995). Mosquitoes can transmit virus transovarially to their progeny to sustain virus in an endemic area (Angel and Joshi, 2008). Transovarial transmission of dengue is a crucial etiological phenomenon responsible for persistence of the virus during inter-epidemic period of the disease (Angel and Joshi, 2008).

2.3.3 The host

After an incubation period of 4-10 days, infection by any of the four virus serotypes can produce a wide spectrum of illness, although most infections are asymptomatic or subclinical Primary infection is thought to induce lifelong protective immunity to the infecting serotype (Halstead, 1974). Individuals suffering an infection are protected from clinical illness with a different serotype within 2--3 months of the primary infection but with no long-term cross-protective immunity. Dengue infections can be life-threatening when they occur in individuals with asthma, diabetes and other chronic diseases (Kouri et al., 1987) (Halstead et al., 1970) (Lee et al., 2006). Individual risk factors determine the severity of disease and include secondary infection, age, ethnicity and possibly chronic diseases (bronchial asthma, sickle cell anaemia and diabetes mellitus). Young children in particular may be less able than adults to compensate for capillary leakage and are consequently at greater risk of dengue shock.

Sero-epidemiological studies in Cuba and Thailand consistently support the role of secondary heterotypic infection as a risk factor for severe dengue, although there are a few reports of severe cases associated with primary infection (Halstead et al., 1970) (Sangkawibha et al., 1984) (Guzman et al., 2000).

The time interval between infections and the particular viral sequence of infections may also be of importance. For instance, a higher case fatality rate was observed in Cuba when DENV-2 infection followed a DENV-1 infection after an interval of 20 years compared to an interval of four years (Guzman et al., 2002). Severe dengue is also regularly observed during primary infection of infants born to dengue-immune mothers. Antibody-dependent enhancement (ADE) of infection has been hypothesized (Halstead, 1989) (Halstead et al., 2005) as a mechanism to explain severe dengue in the course of a secondary infection and in infants with primary infections. In this model, non-neutralizing, cross-reactive antibodies raised during a primary infection, or acquired passively at birth, bind to epitopes on the surface of a heterologous infecting virus and facilitate virus entry into Fc-receptor-bearing cells. The increased number of infected cells is predicted to result in a higher viral burden and induction of a robust host immune response that includes inflammatory cytokines and mediators, some of which may contribute to capillary leakage (Srikiatkhachorn, 2009). During a secondary infection, cross-reactive memory T cells are also rapidly activated, proliferate, express cytokines and die by apoptosis in a manner that generally correlates with overall disease severity (Srikiatkhachorn, 2009). Host genetic determinants might influence the clinical outcome of infection (Kouri et al., 1989) (de la et al., 2007), though most studies have been unable to adequately address this issue. Studies in the American region show the rates of severe dengue to be lower in individuals of African ancestry than those in other ethnic groups (de la et al., 2007)..The dengue virus enters via the skin while an infected mosquito is taking a blood meal. During the acute phase of illness the virus is present in the blood and its clearance from this compartment generally coincides with defervescence. Humoral and cellular immune responses are considered to contribute to virus clearance via the generation of neutralizing antibodies and the activation of CD4+ and CD8+ T

lymphocytes. In addition, innate host defence may limit infection by the virus. After infection, serotype-specific and cross-reactive antibodies and CD4+ and CD8+ T cells remain measurable for years. Plasma leakage, haemoconcentration and abnormalities in homeostasis characterize severe dengue (Srikiatkhachorn, 2009). The mechanisms leading to severe illness are not well defined but the immune response, the genetic background of the individual and the virus characteristics may all contribute to severe dengue. Recent data suggest that endothelial cell activation could mediate plasma leakage (Avirutnan et al., 1998, Cardier et al., 2005). Plasma leakage is thought to be associated with functional rather than destructive effects on endothelial cells. Activation of infected monocytes and T cells, the complement system and the production of mediators, monokines, cytokines and soluble receptors may also be involved in endothelial cell dysfunction (Halstead, 2003). Thrombocytopenia may be associated with alterations in megakaryocytopoieses by the infection of human haematopoietic cells and impaired progenitor cell growth, resulting in platelet dysfunction (platelet activation and aggregation), increased destruction or consumption (peripheral sequestration and consumption). Haemorrhage may be a consequence of the thrombocytopenia and associated platelet dysfunction or disseminated intravascular coagulation. In summary, a transient and reversible imbalance of inflammatory mediators, cytokines and chemokines occurs during severe dengue, probably driven by a high early viral burden, and leading to dysfunction of vascular endothelial cells, derangement of the haemocoagulation system then to plasma leakage, shock and bleeding.

2.4 Pathogenesis and pathophysiology

Most people with dengue fever are asymptomatic with a small percentage having uncomplicated features like fever. Children often present with severe gastroenteritis and other non-specific symptoms such as fever, myalgia and a running nose. However, once infected, they are more

susceptible to severe infection. In Asian countries dengue mostly affects children but, in the Phillipines and Malasyia, a marked increase in DHF has been reported among people of 15 years and above (Pinheiro and Corber, 1997).

Dengue fever is characterised by sudden onset of fever accompanied by headache, pain behind the eyes, generalized myalgia and arthralgia, flushing of the face, rash, anorexia, abdominal pain, and nausea. A febrile phase then follows that is often associated with fevers above 40°C (the fever is biphasic in nature, appearing every one or two days) for 2-7 days accompanied by headaches and generalised pains. At this stage, a skin rash may appear in some patients. Petechiae that are caused by broken capillaries can occur at this stage. Mild bleeding from the mucous membranes of the mouth and nose may also occur. Antibodies directed against Dengue Virus non structural protein 1(NS1) showed cross reactivity with human platelets and endothelial cells, which lead to platelet and endothelial cell damage and inflammatory activation (Lin et al., 2006). A critical phase follows shortly after that has significant accumulation of fluid in the abdominal cavity and in the chest. This is due to the increased capillary permeability and leakage. Organ dysfunction and severe bleeding from the gastrointestinal system may occur followed by shock and haemorrhage. The shock is the Dengue shock syndrome and the haemorrhage is the Dengue hemorrhagic fever. The recovery phase then follows with resorption of the fluid back into the bloodstream. A slow heart rate and severe itching are noted. Fluid overload may occur at this stage and cause cerebral oedema with associated seizures and loss of consciousness (Cook, 2009).

People who have recovered from primary DENV infections develop robust antibody responses that cross react with the 4 serotypes. Despite the cross reactivity, antibodies only prevent re-infection by the same serotype (homologous serotype) and individuals are susceptible to a

second infection with a different serotype (heterologous serotype) (Roehrig, 2003, Rothman, 2003). People experiencing a secondary dengue infection with a new serotype face a much greater risk of developing DHF indicating that pre-existing immunity to DENV can exacerbate disease. Antibody dependent enhancement (ADE) of DENV is the most widely supported theory explaining the higher risk of DHF associated with secondary infection (Halstead, 2003).

Surveillance and diagnosis of this disease has also proved difficult. This is due to poor diagnosis and the non-specific clinical presentation. Clinical management is wanting with lots of room for improvement. Diagnosis has markedly improved with the introduction of dengue specific diagnostic tools. However marked cross reactivity of antibodies within the Flaviviridae family has been a major setback (Blackburn et al., 1995) (Chanas et al., 1979). This serves to give misleading figures on the incidence and prevalence of the disease. These factors serve to confirm the importance of mapping out emerging and re-emerging areas of infection. The development of a suitable vaccine is the mainstay of disease control. There are ongoing vaccine trials that aim to curb spread of this disease.

2.5 Under recognition of Dengue fever

In regions to which malaria is endemic, >70% of febrile illnesses are treated as presumptive malaria, often without proper medical examination and a laboratory diagnosis (Amexo et al., 2004) (Ndyomugyenyi et al., 2007). Many patients in Africa with fever are designated as having fever of unknown origin or malaria and remain without a diagnosis even if they fail to respond to anti-malarial drugs. A study was carried out to ascertain arbovirus co-infection among febrile patients clinically suspected to have either typhoid or malaria in Borno State, Nigeria. Sera samples were tested for *Plasmodium falciparum* by microscopy, *Salmonella Typhi* by Widal and arboviruses: WNV, YFV, DENV and CHIKV using plaque reduction neutralization assay. While 92% of patients tested positive for malaria, typhoid, an arbovirus infection, or a combination of

one or more of these types of infections, less than 1% of the patients tested positive for malaria alone and only 3.9% tested positive for typhoid alone. Approximately half of the patients tested positive for infection with a single arbovirus (48%) regardless of the presence or absence of malaria or typhoid. Of those who showed 90% to 95% virus neutralization, 67.7% had neutralizing antibodies against DENV, 50% against CHIKV, 25% against WNV and 8.7% against YFV (Baba, 2013 #22). Under these prevailing practices, there is a real potential of misdiagnosing dengue as malaria. Except for some reported local outbreaks, many cases of dengue in Africa are more frequently reported among travelers than among the local population, which suggests lack of awareness, diagnostic facilities, and surveillance. In addition, travelers with febrile illness are frequently given a misdiagnosis of malaria; a rate of misdiagnosis as high as 77% has been reported (Amexo et al., 2004).

Of 27 countries in Africa where travelers/expatriates acquired dengue, only 15 have reported local disease transmission. Therefore, travel-acquired dengue appears to serve as a proxy for identifying the under recognition of dengue in Africa (Table 2).

Table: 2 locally and travel acquired, n=15

Angola	1986, 1999-2000 ‡	Unknown
Burkina Faso	1925 1983-1986 2003–2004,§ 2007‡	Unknown 2 Unknown
Cameroon	1987–1993,§ 1999–2002,‡ 2000–2003,§ 2006‡	Unknown
Comoros	1943–1948 1984, 1992–1993†	Unknown 1 and 2
Djibouti	1991–1992†	2
Côte d’Ivoire	1982	2
	1998	1
	1999–2002‡	Unknown
	2008	3
Ghana	1932, 1987–1993§ 2002–2005	Unknown 2
Kenya	1982 1984–1986	2 1 and 2
Madagascar	1943–1948	Unknown

	2006	1
Mozambique	1984–1985†	3
Nigeria	1964–1968	1
Senegal	1979	1
	1980–1985	2 and 4
	1990, 1999	2
	2007‡	Unknown
	2009	3
Somalia	1982, 1985–1987 1992-1993	2 2 and 3
South Africa	1927†	1
Zanzibar	1823, 1870, 2010‡	Unknown

Travel/expatriate, n=12

Benin	1987–1993§	Unknown
DRC	1999–2001,‡ 2007§	Unknown
Ethiopia	1999–2002,‡ 2007§	Unknown
Equatorial Guinea	1999–2002‡	Unknown
Gabon	1999–2002‡	Unknown
Mali	2008	2
Namibia	1999–2002,‡ 2006‡	Unknown
Rwanda	1987–1993§	Unknown
Tanzania	1987–1993,§ 1999–2002,‡ 2006,‡ 2010‡	Unknown
Togo	1987–1993,§ 1999–2002‡	Unknown
Uganda	1999–2002‡	Unknown
Zambia	1987–1993§	Unknown

†Large local outbreaks. ‡TropNet Europ Network and ProMED. §Seroprevalence study.

2.6 Factors Potentially Affecting Sustained Transmission of DENV in Africa

2.6.1Vector Efficiency

Aedes aegypti mosquitoes, the principal DENV vector, originated in Africa and spread to other countries in Africa and other tropical countries in the 17th and 18th centuries (Gubler and Clark, 1995) (Gubler, 2004). Several other *Aedes* species mosquitoes, including *Ae. albopictus*, *Ae. africanus*, and *Ae. luteocephalus*, are found in Africa and are potential DENV vectors. Urbanization is a major factor in facilitating the increase of *Aedes* spp. mosquito populations (Gubler and Clark, 1995). Accumulation of non-biodegradable, human-made containers in and

around living areas has provided the aquatic environment required by these mosquitoes (Monath, 1994). Since the 1950s, a three-fold increase in urban human population density has occurred in Africa; larger increases have occurred in Asia and the Americas (WHO, 2012). With these demographic changes and subsequent increases in *Aedes* spp. populations, increased DENV transmission is likely to occur in Africa. For example, in Ghana, *Aedes* spp. mosquito densities and biting rates seem sufficient to result in outbreaks of yellow fever and dengue (Appawu et al., 2006). Susceptibility of different strains of *Aedes* spp. mosquitoes to DENV has been shown to vary geographically, and this variability may have implications for DENV transmission and the epidemiology of the disease in Africa. Mosquito strains in Africa have shown uniformly low susceptibility to all 4 DENV serotypes in laboratory settings (Gubler et al., 1979) (Gubler and Rosen, 1976). In addition, it has been well documented that there are different susceptibilities of the vector to different DENV genotypes; *Ae. aegypti* mosquitoes tend to be more susceptible to infection with DENV-2 of the Southeast Asian genotype than to the American genotype (Armstrong and Rico-Hesse, 2003). Similar findings have been described for yellow fever virus, and the reduced vector competence of strains of *Ae. aegypti* mosquitoes from Asia has been suggested as an explanation for the absence of this disease in Asia (Gubler, 2004) (Monath, 1989). Reduced vector competence for DENV infection in Africa may be an explanation for some of the apparent low prevalence of DENV infection in Africa, although this explanation must be confirmed in appropriate studies. *Ae. albopictus* mosquitoes are also potential DENV vectors in Africa where they are considered more anthropophilic than *Ae. aegypti* mosquitoes, more susceptible to DENV infection, and are responsible for some dengue outbreaks in Africa (Ratsitorahina et al., 2008, Metselaar et al., 1980). However, similar to studies with *Ae. aegypti* mosquitoes, experimental studies with *Ae. albopictus* mosquitoes have demonstrated that

geographic variations in susceptibility to DENV infection occur among different species (Gubler and Rosen, 1976). Furthermore, *Ae. albopictus* mosquitoes are believed to be less efficient as an epidemic vector largely because of their differences in host preferences and reduced vector competence, which decreases the probability of sustained disease transmission (Lambrechts et al., 2010). Thus, appropriate ecologic studies are needed in Africa to determine the relative roles of each species in transmission of DENV.

2.6.2 Virus Infectivity

Dengue is caused by 4 genetically related but antigenically different viruses, and although it is uncertain where DENV evolved, maintenance of all 4 serotypes in enzootic cycles in Africa suggests that a progenitor virus most likely originated in Africa (Gubler and Clark, 1995). Despite the apparent origin of DENV in Africa hundreds of years ago, the more recent reported outbreaks appear to be the result of virus introductions from Southeast Asia or the western Pacific region and not the result of spillover from forest transmission cycles(Monath, 1994). Vasilakis et al. reported that the rate of evolutionary change and pattern of natural selection are similar among endemic and sylvatic DENVs and suggested possible future reemergence of DENV from the sylvatic cycle (Vasilakis et al., 2007). Recent experimental evidence suggests that emergence of endemic DENV-2 from sylvatic progenitors may not have required adaptation to replicate efficiently in humans, implying that sylvatic DENV-2 may re-emerge (Vasilakis et al., 2007). Existence of a silent zoonotic transmission cycle affords a potential mechanism for emergence of dengue in human populations and for selection of virus variants with altered host range and vector relationships (Monath, 1994).

2.6.3 Host Susceptibility

Host genetic factors influencing pathogenesis have been suggested to account for some variability in susceptibility of DENV infection and disease expression among different races.

Halstead et al. provided evidence of a dengue resistance gene in the black population (Halstead, 1992). During the 1981 and 1997 dengue epidemics in Cuba, blacks were hospitalized with DHF/dengue shock syndrome at lower rates than whites (Guzman et al., 2000). This potential decreased susceptibility to severe disease among the black population and similar observations in Haiti have been used to support the hypothesis that specific genomic difference among different racial groups is a risk factor for DHF (Halstead, 1992) (de la et al., 2007). This hypothesis may provide an explanation for the observation that, to our knowledge, outbreaks of DHF/dengue shock syndrome have not been reported in Africa. Other prevailing diseases in Africa could provide another hypothesis to explain the apparently low incidence of dengue. Malaria, tuberculosis, and HIV infections are endemic to many parts of Africa. Prevailing socioeconomic and environmental factors may make populations in Africa more vulnerable to these diseases than to dengue. Gubler (Gubler, 2004) hypothesized that immunologic cross-protection from heterotypic antibodies to other flavivirus infections (DENV and Japanese encephalitis virus) could explain the absence of yellow fever virus in Asia. A similar argument could be made to explain the low rate of DENV infection caused by cross-protection from other endemic flaviviruses in Africa, but the extent to which it may exist is unknown.

2.6.4 Other Factors affecting transmission

Human activities are also important such as irrigation projects and building of dams that can act to enhance arthropod activity by providing suitable habitat for breeding. Environmental factors such as rainfall, temperature and humidity are very important in the disease transmission process. Global warming with its effects on rainfall, temperature and humidity are playing a very important role in emergence and re-emergence of various diseases. Seasonal variations in the amplitude of daily temperature fluctuations helps to explain seasonal forcing of DENV

transmission at locations where average temperature does not vary seasonally and mosquito abundance is not associated with dengue incidence (Lambrechts et al., 2011). Global warming also affects the various habitats of both humans and vertebrate hosts.

2.7 Immune response to arbovirus infection

2.7.1 Virion and Antigenic Structure

The predominant DENV antigens recognized by antibodies following primary virus infection are E, NS3, and NS5 with a broadened response (E, C, prM, NS1, NS3, and NS5) following re-infection (Churdboonchart et al., 1991, Valdes et al., 2000).

2.7.2.1 Immune Responses: - Innate Immune Responses

It is generally believed that upon release into the skin by a feeding mosquito, DENV replicates in local dendritic cells (Palucka, 2000, Boonnak et al., 2008), with subsequent systemic infection of macrophages/monocytes (Kou et al., 2008, Blackley et al., 2007), followed by entry of the virus into the blood. The liver is also a target organ of DENV, with virus present in hepatocytes, but severe liver disease with jaundice is rare.

2.7.2.2 Adaptive immune responses

The immune response to primary DENV infection is relatively conventional, with an early IgM response to dengue antigens followed later by an IgG response (predominantly IgG1 and IgG3 subclasses) (Koraka et al., 2001). People exposed to DENV infections have detectable specific antibody for decades if not longer. A large fraction of the response cross reacts with all 4 serotypes and even other flaviviruses. In fact the dominance of cross reactive antibodies precludes the use of simple antigen binding assays to identify a flavivirus responsible for infection.

The IgM titres rise at the initial phase of infection but are not good disease markers as they can be missed very easily. The IgG titres rise between days 7-14. Neutralising antibodies are found

within a few days and may persist for years. These neutralising antibodies can cause severe disease through Antibody Dependent Enhancement (ADE) (Boonnak et al., 2008).This phenomenon is best seen in Dengue Haemorrhagic Fever and Dengue Shock Syndrome. Most patients recover with a great residual degree of resistance to members of the same group. This phenomenon is thought to reduce the severity of the disease. The T cell mediated response is not clearly known but may aid in clearing of the infection as well as contribute to the disease process through cytolysis and inflammatory response. The elderly, children and those with pre existing diseases are noted to have severe disease states.

2.8 Diagnostic tools for Dengue Virus infection.

Dengue virus is one of the most important arthropod spread infections globally. It is known to cause many asymptomatic infections and is potentially fatal in severe infections. However, surveillance is poor and not much is known epidemiologically. This is because detection of the viruses in human sera particularly in endemic areas is cumbersome, difficult and also not desirable (Philip Samuel and Tyagi, 2006). These factors serve to compound an already dire situation.

Laboratory confirmation of dengue virus infection is important. Although in practice diagnosis is often made by clinical signs and symptoms only, dengue can be confused clinically with other vector-borne viral and parasitic diseases, such as malaria, chikungunya and zika viruses.

Diagnostic tools available are also not reliable as there is marked cross reaction of the antibodies across the Flaviviridae family noted (Blackburn et al., 1995) (Chanas et al., 1979).

Diagnosis may involve detection of the virus, viral nucleic acid, antigens or antibodies, or a combination of these entities. Laboratory tests using NS1 (non-structural protein 1) antigen can provide early diagnosis in febrile patients. After the onset of illness, the virus can be detected in serum, plasma, circulating blood cells and other tissues for 4<5 days. During the acute stage of

the disease, virus isolation, nucleic acid or antigen detection can be used to diagnose the infection. At the end of the acute phase of infection, serology is the method of choice for diagnosis. Serological assays to detect specific immunoglobulin M (IgM) or immunoglobulin G (Huang et al.) antibodies to dengue virus are widely available.

Many studies have attempted to find a reliable diagnostic tool. Several serological and virological diagnostic tools for dengue infection exist. They are haemagglutination inhibition, MAC-ELISA, plaque reduction neutralisation test and indirect immunofluorescence antibody assay (De Paula and Fonseca, 2004). Some studies use mosquitoes as the test sample while other studies use infected human specimens. A study was done that applied a rapid and sensitive, semi-nested, reverse transcription PCR (RT PCR) assay using NS3 gene primers for the type specific detection of dengue virus in artificially infected and field caught *Aedes* mosquitoes (Chow et al., 1998). In laboratory experiments that were done, the assays were sensitive enough to detect one virus infected mosquito head in a pack of up to 59 infected heads. Virological surveillance of the dengue virus infected *Aedes* mosquito may serve as an early warning monitoring system for dengue outbreaks (Chow et al., 1998). Another study compared the nested and the Fourplex Real Time Reverse Transcriptase Polymerase assay. The nested method is a two step method that has increased chances for contamination. It takes about 10 hours to run and many reactions are needed to identify the serotypes. The Fourplex system, a closed system with less contamination takes 3 hours to visualise and identifies all the Dengue Virus serotypes at once. The study showed the superiority of the Fourplex assay in both diagnosis and identification of the viral serotypes (Johnson et al., 2005). Another study compared IgM Capture ELISA with a commercial rapid immunochromatographic card test. They also assessed IgM microwell ELISA for the detection of antibodies to dengue virus. Samples from patients with febrile-like illnesses

and symptoms resembling Dengue fever were then processed. The positive rates were then noted. MAC-ELISA had 38%, immunochromatographic card test had 22.7% and Pan Bio IgM ELISA had 20.7%. Sensitivity rates were also compared with MV MAC-ELISA at 96%, Pan Bio Rapid at 73% and Pan Bio IgM ELISA at 72%. The study noted that MAC-ELISA had a high sensitivity and specificity (Sathish et al., 2002). Better diagnosis, surveillance, prevention, predictive models and improved understanding of the spread of dengue to previously uninfected regions in the context of globalisation and climatic changes would help control spread of the disease (Wilder-Smith et al., 2012).

3.0 RESEARCH DEFINITION

3.1 Justification

Improved transport systems and excellent communication channels mean that the world has become a global village. Improved global transport networks therefore makes it easy for the spread of infectious diseases (Tatem et al., 2006). A rise in rural to urban migration together with increased populations in Kenyan towns has led to an increase in both permanent and semi permanent housing structures. The vector that transmits Dengue fever *Ae. aegypti* thrives in such conditions especially in the tropical and the subtropical areas. The improved transportation systems means that infected people can move with ease and transmit the disease. This disease has been noted to be on the upward trend globally (Kyle and Harris, 2008). Kenya has poor surveillance systems that make the country vulnerable and open to outbreaks. Dengue fever mainly presents as a febrile illness with various non-specific signs and symptoms. It is therefore misdiagnosed or missed altogether (Amexo et al., 2004). Between 250 000 and 500 000 people develop severe dengue each year (Deen et al.). A few local studies did show an emergence and re-emergence of arboviral infections in various Kenyan locations (Sang and Dunster, 2001). Nationwide studies need to be done to establish the hotspots and highlight the emerging areas.

The choice of NBTS as a study site is because; blood donors are considered a low risk population, which is the reason why they qualify to donate blood for transfusion purposes. Nairobi, Eldoret and Kisumu regional blood transfusion centres were selected as sites for this study because, Kenya has had reported outbreaks mainly in the coastal region with no reported outbreaks in other regions of the country. Therefore it was necessary to find out whether Dengue virus is circulating in these parts of the country so that this data can be used to inform policy.

3.2 Research Question

1. Are there dengue fever virus hotspots in Kenya?

2. What is the prevalence of Dengue fever virus in the adult Kenyan population in Nairobi, Eldoret and Kisumu?

3.3 Objectives

Broad objectives: To determine dengue fever virus hotspots in selected regions in Kenya.

Specific objectives:

1. To determine the hotspots of dengue fever virus in some parts in Kenya.
2. To determine the prevalence of Dengue fever virus in the selected areas in Kenya.

4.0 METHODOLOGY

4.1 Study period and study design

The study was conducted between June 2013 and June 2014. The study was a cross-sectional descriptive study.

4.2 Study population

The study recruited consenting adults donating blood at the regional blood transfusion centres (RBTCs) in Nairobi, Eldoret and Kisumu.

Inclusion and exclusion criteria

Study participants were consenting adults above 18 years of age and from the selected regions. People who were unable to give consent and those below 18 years were excluded from the study.

4.3 Study site

The study was carried out at three Regional Blood Transfusion Centres (RBTCs) of the National Blood Transfusion Services (KNBTS) in Nairobi, Eldoret and Kisumu in Kenya. The RBTCs have large catchment areas from where they get their blood. Nairobi and Eldoret are highland areas while Kisumu is a lowland area of Kenya. The samples were processed at the RBTCs laboratories in Kisumu and Eldoret, where the separation of blood into plasma was done before shipping to UNITID laboratories. Nairobi samples were processed at UNITID laboratories.

4.4 Sample size and sampling method

Sample size calculation Formula:

The sample size was estimated according to Fisher's formula (Fisher 1991).

$$n = \frac{t^2 \times p(1-p)}{m^2}$$

Description:

n = required sample size for this study.

t = t statistic for a level of confidence level at 95% ($\alpha/2$ standard value of 1.96)

p = expected prevalence or proportion or estimated proportion of dengue. That is the prevalence that will be estimated in this study i.e. 50% (0.5)

m = margin of error at 5% (standard value of 0.05)

$$N= \frac{1.96^2 \times 0.5(1-0.5)}{0.05^2} = 1.96^2 \times 0.25 / 0.0025$$

N=<u>384</u>

A sample size of **384** was arrived at using the given information above. This is the minimal necessary number to achieve the required sufficient precision for the estimated prevalence.

Sampling method

For this study a sample size of 490 willing adults were recruited upon signing a written Informed consent form. All the adults who met the inclusion criteria were identified from the rest of blood donors. After identification, the potential study participants were taken through the informed consent process whereby the study objectives, risks, benefits and study procedures were explained in Kiswahili, English or a language of the participant's preference.

Systematic random sampling method was used to recruit study participants. A total of 200 study participants were selected from Nairobi, 140 from Eldoret and 150 from Kisumu regions (The regional numbers were randomly assigned). The study participants were recruited using the formula below: - an average of 40 people attends blood donation services per day. Sample collection period in a region was approximately 14 days.

———————=14 per day.

———————————————— =3;

Therefore from the formula every third person was recruited in to the study. If the person was not willing the fourth person was recruited.

4.5 Data collection

After obtaining informed consent, the study participants were taken through an interview during which a questionnaire was used to obtain the socio-demographic information and past health history as well as an assessment of epidemiological risk factors. Each participant was interviewed in private to promote confidentiality.

Laboratory results from the tests were entered into records with the same number (code) as the one on the questionnaires. All the data were entered into MS Excel and later exported into SPSS for analysis and drawing of conclusions.

4.6 Sample collection and sample processing

2mls of blood were collected in to sterile EDTA Blood vacutainers from consenting individuals during blood collection at the regional blood transfusion centres. The blood was from the blood bag therefore the participant was not pricked twice. Each bottle was labelled with the participant's number (code) as recorded in the questionnaires. The blood collected was transported in a cool box to the laboratory. The samples were kept in 2-8°C for 24 hours then centrifuged to obtain 1000ul plasma which was then frozen at -80°C till all the samples had been collected and ready to be used. The samples were then shipped to the National Microbiology Laboratory (NML) in Canada to be tested for IgG antibodies using ELISA assays.

4.6.1 Laboratory assays

Dengue Virus IgG DxSelect™ ELISA Kits (product code EL1500G, Focus Diagnostics).

The Dengue DxSelect™ IgG ELISA kits is designed for optimal performance and cost efficiency. The kit utilizes inactivated dengue virus types 1-4 and contains 96 breakaway wells to give laboratories the flexibility to set up runs based on volume requirements, improve turnaround time with reduced incubation requirements, and eliminate sample pre-treatment. The Focus Diagnostics Dengue Virus ELISA IgG is an indirect ELISA intended for the detection of

antibodies to DENV types 1-4. This assay is specific for IgG and uses a horse radish peroxidase conjugate with TMB as substrate. Each antigen coated well contains equal proportions of inactivated, purified DENV types 1-4. The following virus strains were used: Type 1: TH-Sman; Type 2: TH-36, Type 3:H87; and Type 4: H241.The Focus Diagnostics Dengue Virus ELISA IgG has a sensitivity of 98% for DF, 93% for DHF and an overall specificity of 93%.

Indirect IgG ELISA

Test principle

In the Focus Diagnostics Dengue Virus IgG ELISA assay, the polystyrene microwells are coated with equal proportions of inactivated and purified Dengue virus types 1-4. Diluted serum samples and controls are incubated in the wells to allow specific antibody present in the samples to react with the antigen. Non-specific reactants are removed by washing and peroxidase-conjugated anti-human IgG is added and reacts with specific IgG. Excess conjugate is removed by washing. Enzyme substrate and chromogen are added, and the color is allowed to develop. After adding the Stop Reagent, the resultant color change is quantified by a spectrophotometric reading of optical density (OD) which is directly proportional to the amount of antigen-specific IgG present in the sample. Sample optical density readings are compared with reference cut-off OD readings to determine results.

Procedure

Each specimen, control and calibrator 1:101 was diluted as follows: the tubes were labeled and 1000 µL of Sample Diluent was dispensed into each labeled tube. 10 µL of specimen, control or calibrator was then added to each appropriate tube containing the 1000 µL Sample Diluent and mixed well by vortex mixing.

The wells were filled with 1X Wash Buffer solution and allowed to soak for 5 minutes. The antigen wells were aspirated and tapped vigorously to remove Wash Buffer. The emptied antigen

wells were blotted face down on clean paper towels or absorbent paper to remove residual Wash Buffer.

100 µL of the Sample Diluent was dispensed into the "blank" wells and 100 µL of each diluted specimen, control or calibrator into the appropriate wells. The plates were covered with sealing tape, and incubated for 60 ± 1 minute at room temperature (20 to 25°C). The sealing tape was removed, and the contents of the wells emptied into a sink. Each well was filled with a gentle stream of 1X Wash Buffer solution then contents emptied into a sink. The wash steps above were repeated an additional 2 times, the last wash was allowed to soak for 5 minutes before decanting or aspirating. The antigen wells were tapped vigorously to remove 1X Wash Buffer. The emptied antigen wells were then blotted face down on an absorbent paper to remove residual 1X Wash Buffer. 100 µL Conjugate was then dispensed into all wells, using a 100 µL 8- or 12-channel pipettor.

The plates were covered with sealing tape and incubated for 30 ± 1 minutes at room temperature (20 to 25°C).

The three wash steps above were then repeated. 100 µL of Substrate Reagent was pipette to all wells, using a 100 µL 8- or 12-channel pipettor. The incubation timing begun with the addition of Substrate Reagent to the first well. It was then incubated for 10 ± 1 minutes at room temperature (20 to 25°C). The reaction was stopped by adding 100 µL of Stop Reagent (1M Sulfuric acid) to all wells using a 100 µL 8- or 12-channel pipettor. The Stop Reagent was added in the same sequence and at the same pace as the Substrate was added. In antibody-positive wells, there was colour change from blue to yellow. The outside bottom of the wells was gently blotted with a paper towel to remove droplets that may interfere with reading by the spectrophotometer. The absorbance of each well was measured within 1 hour of stopping the

assay. The microwell spectrophotometer was set at a wavelength of 450 nm. The instrument was zerod on the blank wells.

4.7 Data analysis

Data was captured into the questionnaire by the principal investigator and a study assistant. All the questionnaires were reviewed by the principal investigator to ensure they are completed appropriately. Errors were corrected and those that could not be corrected were excluded from the analysis. The data was coded and entered into an Excel spreadsheet in a password protected computer. Back-up copies were stored in an external hard drive and compact disc which were in sole custody of principal investigator.

The filled questionnaires were kept in the safe custody of the principal investigator who filed and stored them in a locked cabinet for verification during analysis. Statistical package for social scientists (SPSS) was used for statistical analysis.

Plasma samples were tested for the presence of IgG antibodies against Dengue fever virus using in-house ELISA IgG assays to determine the prevalence rates.

4.8 Expected outcomes

It is expected that this study will lead to a better understanding of the presence and impact of Dengue fever infections in Kenya.

4.9 Ethical considerations

Permission to do the study was sought from the Kenyatta National Hospital-University of Nairobi Ethical Review committee (reference number P64/02/2013), the Director-Kenya National Blood Transfusion Service and the Regional Directors-Nairobi, Eldoret and Kisumu RBTCs before beginning the study. Informed consent was obtained based on non-coercive approach from the study participants and remained confidential. The study participants were taken through a process of obtaining informed consent whereby the study objectives, process,

risks and benefits were thoroughly explained to them before being enrolled into the study. Dignity of the study participants was upheld throughout the study. The study questionnaires were stripped off all identifying data to maintain participant's confidentiality. A number was assigned to each study participant that was used on the questionnaire and on the sample collected.

Potential risks for the study were minimal, commensurate with routine interrogation and blood tests. They included time considerations and mild pain at the site of puncture for specimen collection.

5.0 RESULTS

A total of 490 blood samples were collected from consenting study participants out of which 387 plasma samples were analysed using indirect IgG ELISA from three RBTCs in Nairobi, Eldoret and Kisumu. Nairobi RBTC covers the whole of Nairobi County, Machakos RBTC satellite which encompasses Kajiado, Kitui, Makueni and Machakos Counties, KIambu county, and Northeastern satellite RBTC which includes Wajir, Mandera and Garissa counties. Eldoret RBTC covers the whole of North Rift and parts of Kakamega and Bungumo counties. Kisumu RBTC covers the whole of Nyanza (except Kisii and Nyamira), parts of Busia and Vihiga counties. A total of 99 samples were from Nairobi, 150 from Kisumu and 138 from Eldoret region. The numbers were assigned randomly to each region.

Section A: Socio-demographic

The median age was 25 with the youngest study participant being 18 and the oldest 64 years.

Figure 5. **Gender**: The figure shows a representation of the gender of the study population. The proportion of females in the study was 16% (n=61) while men was 84% (n=326). 3.3% of the females tested positive for dengue virus antibody while 6.1% of the males were positive.

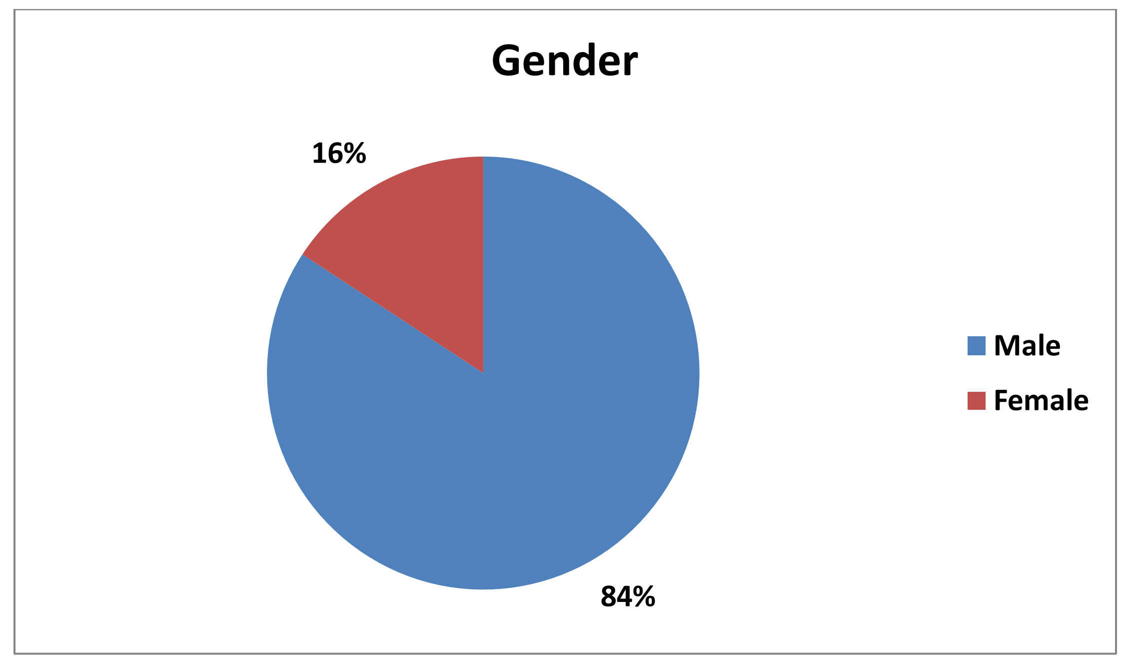


***Figure 6*. Level of Education**: The graph shows the level of education of the study participants. The Y-axis shows the level of education (none, primary, secondary and tertiary) while the x-axis shows the percentages. More than half of the study participants had tertiary level of education at 56.6%.

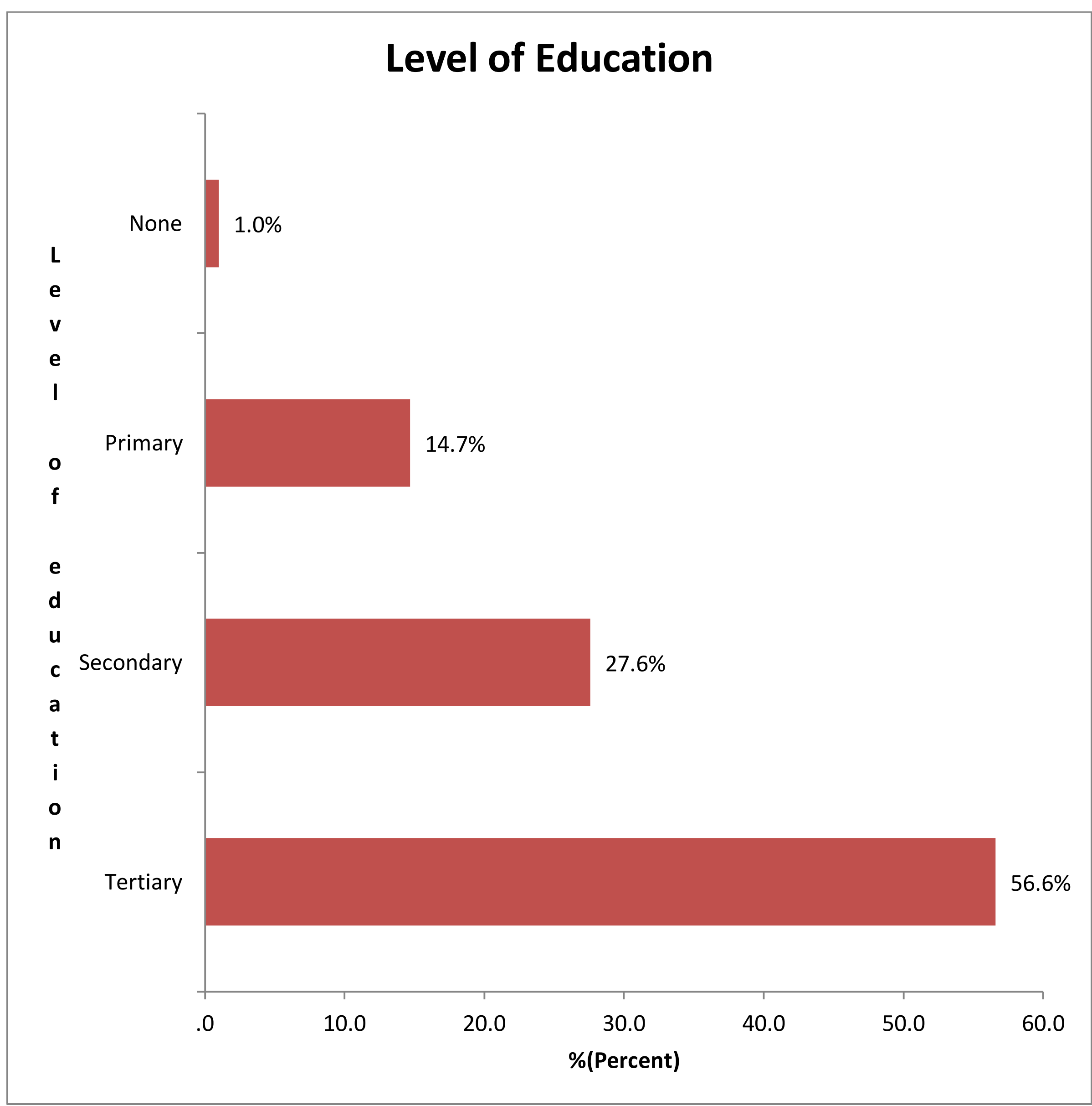
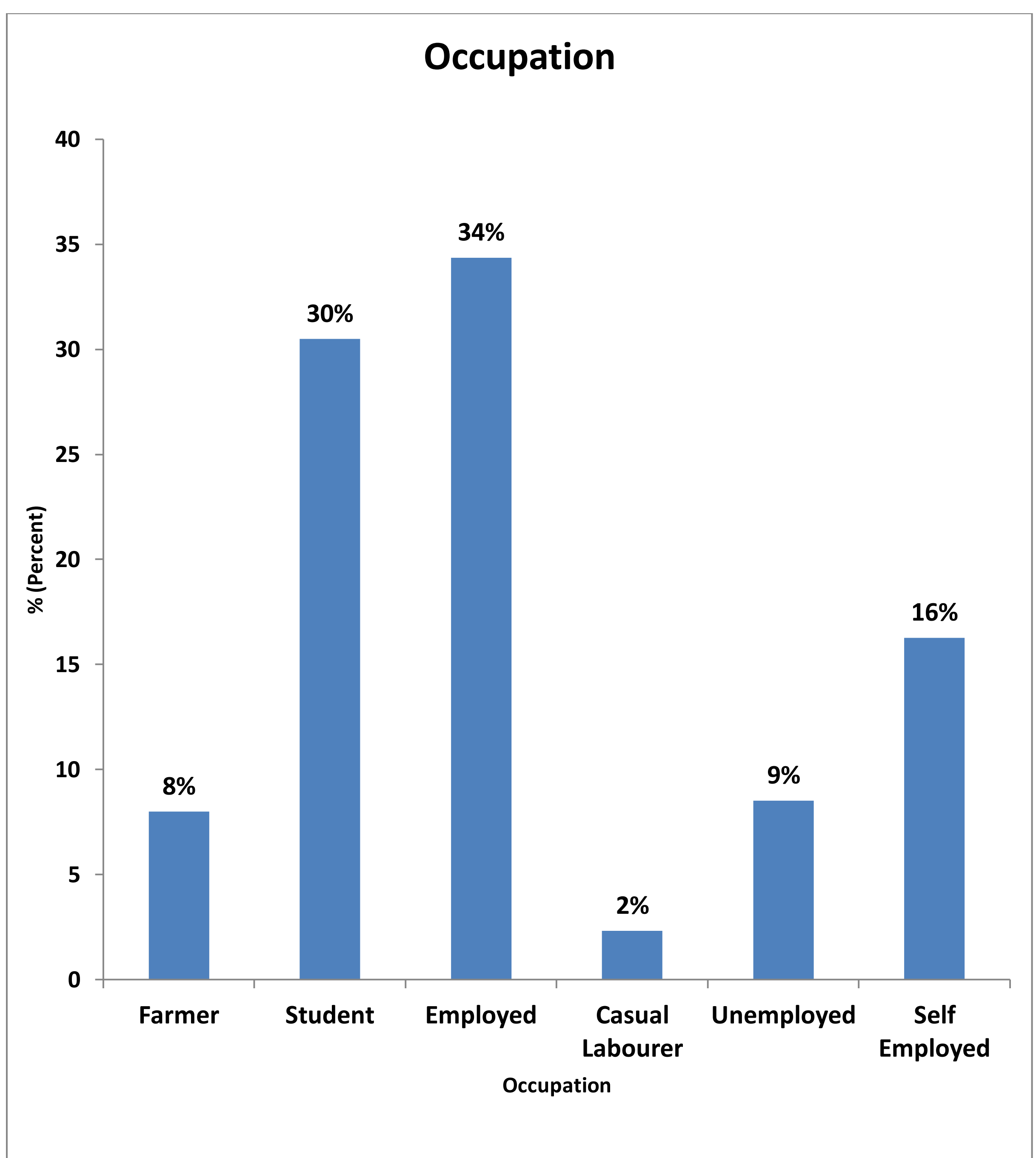


Figure 7. **Occupation**: This graph shows the occupation of the study participants. The y-axis shows the percentage while the x-axis shows the type of occupation of the study participants.Most participants were either students or employed.



Section B: Symptom history

Figure 8. **Fever**: A total of 18% of the population had had fever in the last 6 months while 82% had not had fever. Of the 18% who had fever 7.1% tested positive for dengue; while 5.4% of those who had not had fever also tested positive for dengue.

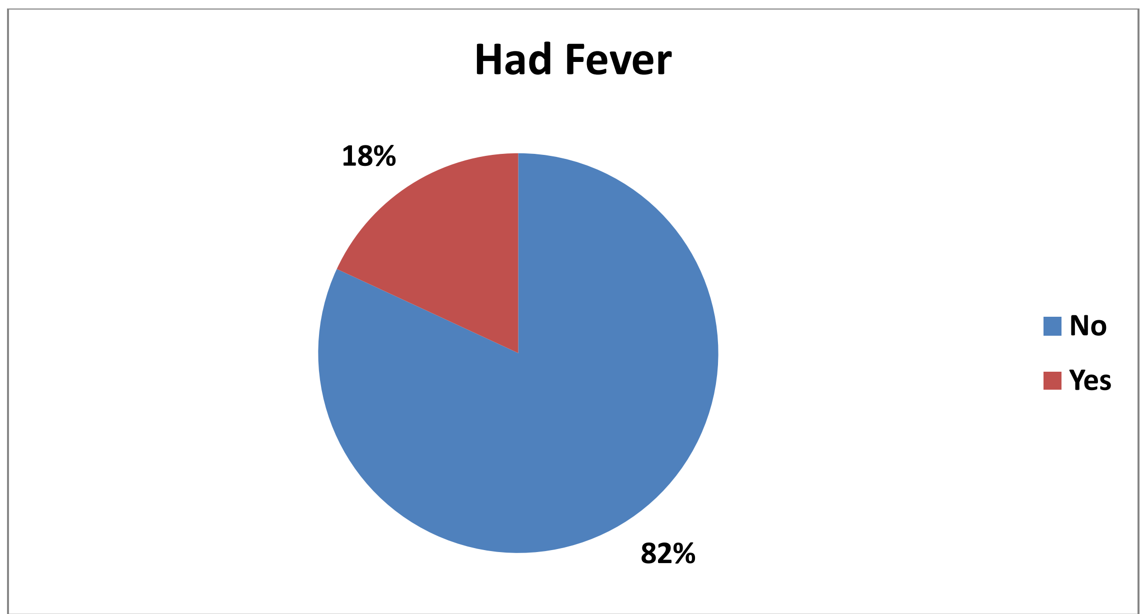
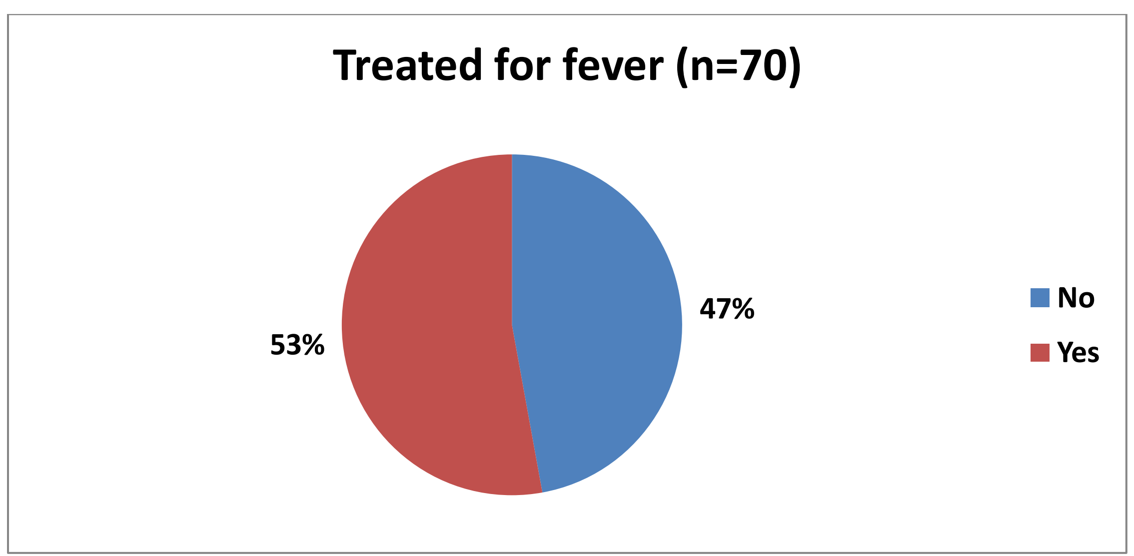


Figure 9. **Treated for fever**. 53% of the 18% who reported to have had fever in the last 6 months sought treatment for fever while 47% of them did not seek treatment.The 47% reported that the fever went away without any medication.



Section C: Risk Factors

***Figure 10*. Use of mosquito**. 65% of the study participants reported to using treated mosquito nets at night always. 35% were not using treated mosquito nets at all.

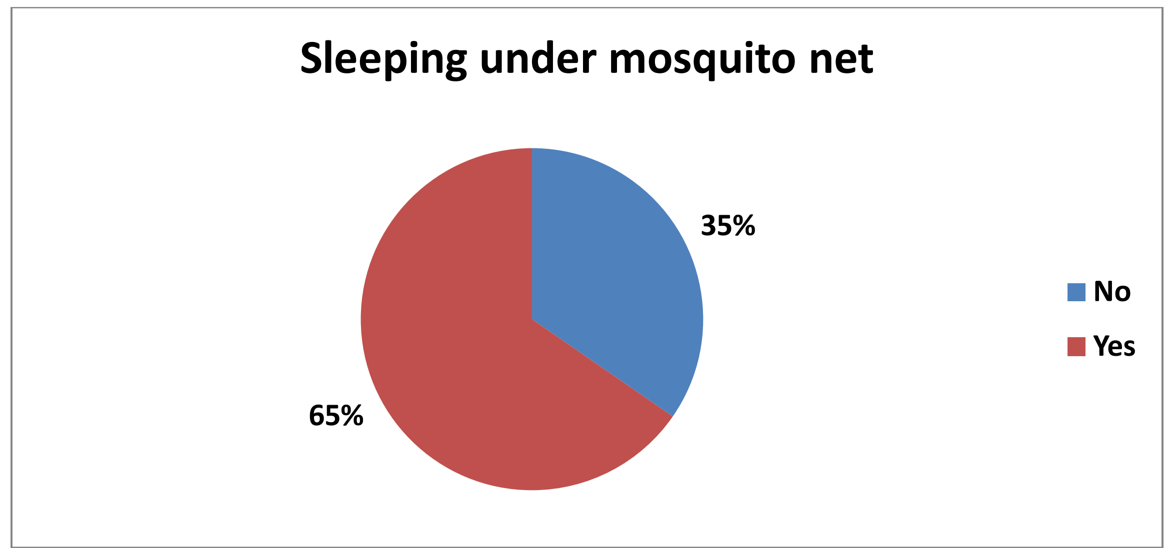
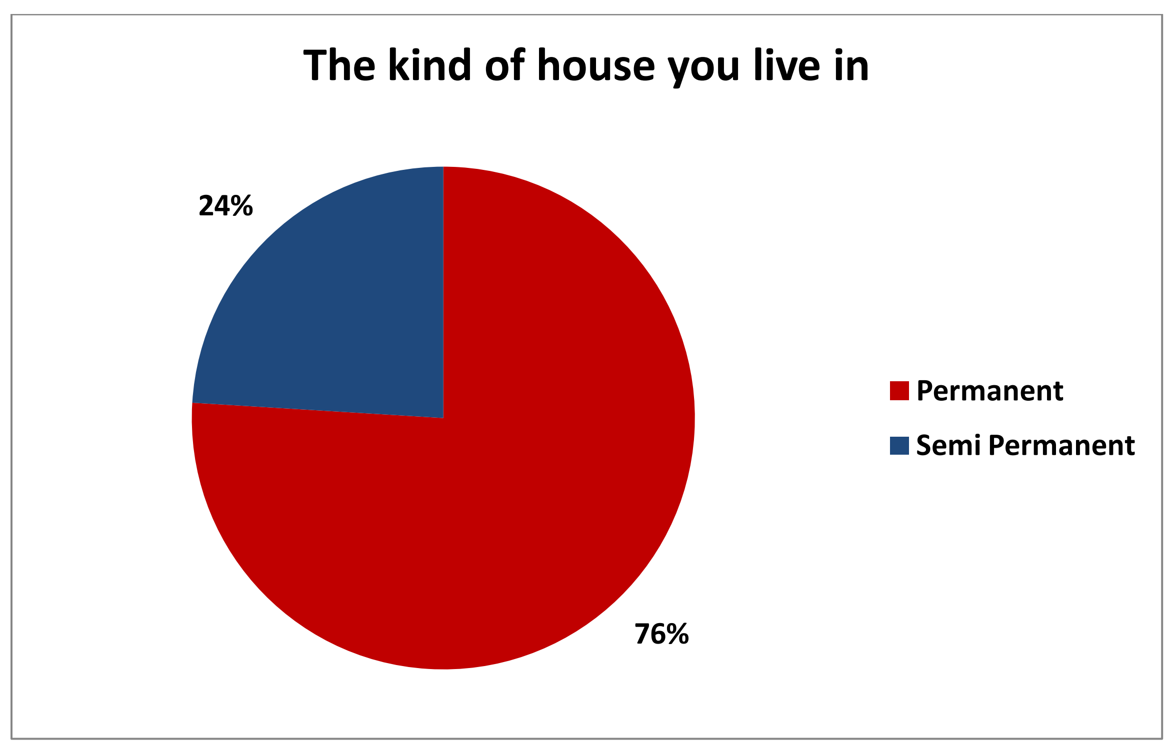


***Figure 11*. The kind of house that the participant lived in**. 76% of the study participants lived in permanent buildings (made of permanent house wall materials), while 24% lived in semi-permanent buildings (made with temporary house wall materials).



Prevalence

The overall prevalence of dengue fever virus in the adult Kenyan population in the three regions was 5.7% (22/387).

Table 3: Prevalence of Dengue Fever Virus in different regions

Region	N	Prevalence
Nairobi Region	99	11(11.1%)
Kisumu Region	150	8(5.4%)
Eldoret Region	138	3(2.2%)

Table 4: Hotspots of Dengue Fever Virus in Kenya

Region	Area of Residence	Number positive for dengue virus	N
Nairobi Prevalence=11.1%	Eastleigh	1	3
	Kariobangi South	1	2
	Kileleshwa	1	2
	Pangani	1	5
	Parklands	1	1
	Rongai	1	22
	Kware	1	3
	South B	1	5
	Westlands	1	2
	Yaya	1	1
	Bulla Jamhuria	1	1
Kisumu Prevalence=5.4%	Arina	2	3
	Makasembo	1	1
	Riat	1	1
	Urudi secondary	1	14
	Manyatta	2	17
	Kisumu town	1	12
Eldoret Prevalence=2.2%	Kapsoya	1	4
	Webuye	1	2
	Eldoret town	1	18

The table below shows counties within the three RBTCs with the highest prevalences. Nairobi county for instance, had the highest prevalence; 8/53 (11.1%).

Table 5: Counties with the highest prevalences

Counties	Prevalences	N
Bungoma	1(33.3%)	3
Kajiado	2(6.9%)	29
Kisumu	8(6.8%)	118
Nairobi	8(11.1%)	53
Uasin Gishu	2(2.4%)	84

Table 6: Data analysis

Characteristics	Dengue fever virus prevalence		Univariate	
	Positive	Negative	OR[95% CI]	p-value
	n (%)	n (%)		
Age (years)				
18-24	8(4.8)	160(95.2)	1	
25-34	10(6.9)	134(93.1)	0.63(0.13-3.11)	0.57
35-44	2(4.2)	46(95.8)	0.93(0.19-4.52)	0.93
>=45	2(7.4)	25(92.6)	0.55(0.07-4.10	0.54
Gender				
Male	20(6.1)	306(93.9)	0.52(0.12-2.28)	0.55
Female	2(3.3)	59(96.7)		
Had Fever				
Yes	5(7.1)	65(92.9)	1.36(0.48-3.81)	0.56
No	17(5.4)	300(94.6)		
Sleep under Mosquito net				
Yes	14(5.5)	239(94.5)	0.92(0.38-2.26)	0.86
No	8(6.0)	126(94.0)		

The table shows the relationship between the different characteristics measured in the study with testing positive for dengue fever virus IgG antibodies.

6.0 DISCUSSION

The study analyzed the presence of anti-DENV 1-4 in plasma of a cross-section of consenting healthy blood donors in three RBTCs using an in-house-Indirect IgG ELISA. The indirect anti-DENV IgG ELISA is often used for epidemiologic studies to determine past DENV exposure because of its improved sensitivity (98%) and specificity (93%) compared to the direct ELISA method. The direct IgG ELISA requires purified antigen, and this reagent is not always available.

The overall prevalence of DENV1-4 in this study population was found to be at 5.7% (22/387); with 11.1% (11/99) in Nairobi region, 5.4% (8/150) in Kisumu region and 2.2% (3/138) in Eldoret region.

There appears to be an increasing magnitude of dengue virus activity in Kisumu region from 1.1% to 5.4% since the last study had found a seroprevalence of 1.1% (Blaylock JM, 2011). This was the first study to document dengue fever virus in Nairobi and Eldoret regions as high as 11.1% in Nairobi and 2.2% in Eldoret. This therefore means that there is dengue fever virus activity in these three regions though no outbreaks have ever been reported.

Out of a total of 18% (70) of the study participants who reported that they had had fever in the last 6 months, only 7.1% (5/66) of them tested positive for anti-dengue IgG. From 82% (317) who reported to have not had fever in the last six months, 5.4% (17) tested positive for dengue. This may be partly due to the fact that most dengue fever infections are asymptomatic (Halstead, 1974).

A total of 65% of the participants reported to consistently using a treated mosquito net at night out of which 5.5% (14) tested positive for DENV. The rest, 35% reported to not using a mosquito net at all out of these, 6.0% tested positive for DENV. This data shows that the use of mosquito net at bed time may not be important in dengue fever prevention since there is no

statistical difference (p-value=0.86) between those who slept under a treated mosquito net and those who did not use one.

The knowledge of dengue fever hotspots in Kenya was key in this study because it is useful in designing prevention and control strategies. The hotspots were selected as areas with the highest numbers of positive samples. Therefore the hotspots of dengue fever virus in Kenya are: Arina, Urudi, Manyatta, Riat, Makasembo in Kisumu region; Eastleigh, Kariobangi South, Kileleshwa, South B, Parklands, Pangani, Kware, Rongai, Yaya and Westlands in Nairobi and Kapsoya, Eldoret town and Webuye in Uasin Gishu County in table 4.

Manyatta and Kware are slum areas having insufficient sewage disposal mechanisms therefore providing suitable habitat for the vector since *Aedes aegypti* can breed well in organically polluted waters. Lack of garbage collection systems especially in most hotspots like Rongai, Eastleigh, Pangani and Eldoret town provides mosquito breeding sites. *Aedes aegypti* is a highly domesticated mosquito that breeds in artificial containers such as water storage tanks, subterranean pits, flowerpot trays and other ornamental containers.

7.0 CONCLUSIONS

The specificity of the In-House-ELISA used was high at 93% for both dengue fever and DHF. The prevalence of dengue fever virus in Nairobi is 11.1%, Kisumu 5.4% and Eldoret 2.2%. These three regions in Kenya have never had reported dengue outbreaks yet this study found that there is dengue virus activity. The presence of anti-dengue IgG among a low risk population (blood donors) therefore shows that there is exposure of the general Kenyan population to dengue virus and hence vulnerability to dengue outbreaks. There is evidence of increase in DENV prevalence in Kisumu region from 1.1% to 5.4% suggesting an increase in vector density due to population growth that leads to creation of breeding sites for *Aedes* sp- a container breeder. The results provide evidence for the first time of dengue activity in Nairobi and Eldoret regions.

8.0 RECOMMENDATIONS

It is evident from this study that dengue has spread to Kisumu, Eldoret and Nairobi therefore:

- Dengue fever virus should be diagnosed among other febrile illnesses in the country.
- Blood donors should be screened to minimize the risk of transfusing contaminated blood especially when such blood or blood components can be used in management of severe dengue cases.
- Emphasis should be placed on mosquito control strategies such as reduction of breeding sites and use of mosquito repellants bearing in mind that *Aedes aegypti* bite at dawn and dusk.
- More studies should be done to determine the circulating serotypes in our population.
- Improved surveillance would serve to predict the peak infection rates.

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APPENDICES

Appendix 1: Information to Participants and Consent Form

TITLE: Seroprevalence of Dengue Fever Virus In The adult Kenyan Population in Nairobi, Eldoret and Kisumu Regions

STUDY SITES: Nairobi, Eldoret and Kisumu

Investigator: Betty J Koech

Supervisors: Prof. Omu Anzala

Dr. Julius Oyugi

Hi. My name is Betty Jepkurui Koech, a postgraduate student at the University of Nairobi. I wish to invite you to take part in this research. The information in this document is meant to help you make a decision.

PURPOSE OF THE STUDY

In this study we are interested in knowing the prevalence and hotspots of Dengue fever virus in the selected areas in Kenya. The study will be conducted in two Regional Blood Transfusion centres with cooperation and permission from National Blood Transfusion Centres, its staff and supervision from the University of Nairobi.

Procedures: - You are being asked to take part in this study that will take you 30 minutes. If you agree to participate, I will ask you some questions and note your responses in writing. Some questions will be personal, but the answers to the responses will remain confidential.

Risks:- Other than the discomfort and pain in obtaining the blood sample, there are no other foreseeable risks that will arise from participating in the study.

Benefits:- By choosing to participate in this study, you will not have any direct benefits from it other than that of a free test to know your health status. However the information obtained from the study will be useful to the country in general by giving information on the disease status of the people and can be used in planning for controlling the spread of Dengue fever virus.

Costs and Compensation: - By choosing to participate in the study, you will not incur any extra monetary cost. You will however take about thirty minutes longer than your usual blood donation time to go through the study procedures. You will not be paid for taking part in the study.

Voluntary participation: - Taking part in this study is voluntary. You have the right to choose not to take part in this study.

Questions:- If you have any questions about this study now or later, you may contact the Principal Investigator, Betty Koech on the following phone number **0720828217 or email:-** bettykoech2004@yahoo.com

If you have any questions regarding your rights as a study participant please contact the KNH/UON ERC Chairperson, Prof. Guantai at phone number:- (254-020) 2726300 ext 44355 or email uonknh_erc@uonbi.ac.ke

Consent form: - Kiswahili version

Habari za leo,

Jina langu ni Betty Koech, kutoka chuo kikuu cha Nairobi. Ninafanya utafiti kwa watu wazima wanaohudhuria mpango wa kutoa damu. Watakao kubali kushiriki itawabidi watie saini au alama ya kidole kwenye fomu kuonyesha kukubali kwao na pia,tutawahoji na kuwapima.

Faida utakayopata katika utafiti huu ni kujua hali yako ya afya.

Taarifa zote utakazotupatia ni siri na zitatumi ka tu kwaajili ya kuboresha huduma ya afya nchini Kenya. Ushiriki wako ni kwahiari kabisa na pia unayo haki ya kujiondoa katika utafiti huu wakati wowote ule utakapojisikia kufanya hivyo. Uamuzi wako wakushiriki katika utafiti huu, hautaadhiri hata kidogo haki yako kushiriki katika zoezi la kutoa damu.

Nitafurahi ukikubali kushiriki katika utafiti huu.

Bi Betty Koech sahihi **_____________________Mtafiti.**

Nani wa kuwasiliana.

Kama una swali lolote juu ya utafiti huu unatakiwa uwasiliane na mtafiti mkuu, Bi Betty Koech Namba-0720 828217 au kwenye barua pepe bettykoech2004@yahoo.com.

Ukiwa na maswali kuhusu haki zako kama mshiriki katika utafiti huu, waweza kuzungumza na mwenyekiti wa KNH/UoN ERC, Profesa Guantai kwenye nambari za simu (254-020) 2726300 ext 44355 au kwenye barua pepe uonknh_erc@uonbi.ac.ke

Statement of Consent

If you agree to participate in this study, please sign below.

I, ____________________________ have read or have had read to me the consent form for the above study. I was free to ask any questions and they have been answered. I am exercising my free power of choice, and hereby give my consent to be included as a participant in this study of 'The prevalence of Dengue fever virus in the Kenyan population.

Participant's Name ____________________________ Signature _____________ Date__/__/____

Research's Name_____________________________ Signature___________ Date__/__/_____

Appendix 2: Questionnaire

To determine the seroprevalence and hotspots of dengue fever virus in Kenya.

Date of data collection (tarehe ya kuchukua damu): _____/______/_________/ (dd/mm/yyyy)

Respondent code (nambari la mshiriki) ______________Study Site:

☐ Nairobi region ☐ Eldoret region ☐ Kisumu region

Section A: Socio-demographic

1. Date of birth (siku ya kuzaliwa)______/_______/_______/ (dd/mm/yyyy)
2. Sex (Jinsia) Male/Mwanamume ☐ Female/mwanamke ☐
3. In which county do you live now?/ Unaishi katika jimbo lipi kwa sasa _______________
4. In which place do you live? /Unaishi katika sehemu gani? _________
5. Duration at present place of residence/ Umeishi hapa kwa muda upi?

   1 month ☐ mwezi mmoja

   Less than 1 year ☐ chini ya mwaka mmoja

   1 year ☐ mwaka mmoja

   5 years ☐ miaka mitano

   More than 10 years ☐ zaidi ya miaka kumi

6. Occupation/ kazi unayofanya

   a) Farmer ☐ Mkulima

   b) Student ☐ Mwanafunzi

   c) Employed ☐ Kazi ya kuajiriwa

   d) Casual labourer ☐ Kibarua

   e) Unemployed ☐ Sina kazi

   f) Self employed ☐ Kazi ya kujiajiri

7. Level of education/kiwango cha elimu

   a) None ☐ Hakuna

   b) Primary ☐ Shule ya msingi

   c) Secondary ☐ Shule ya upili

   d) Tertiary ☐ Elimu ya juu

Section B: Symptom history

8. Have you had fever in the last 6 months/ je umekua na wingi wa joto katika miezi sita iliyopita? Yes/ Ndio ☐ No/Hapana ☐

9. If yes did you go to hospital? / je, ulienda hospitalini kutafuta matibabu?

   Yes/Ndio ☐ No/hapana ☐

10. Did the fever go on its own/ je joto hilo lilienda pekee? Yes/Ndio ☐ No/hapana ☐

Section C: Risks

11. Do you sleep under a treated mosquito net/ Je, unalala chini ya neti lililotibiwa?

    Yes/Ndio ☐ No/hapana ☐

12. The kind of house you live in/ Je unaishi nyumba aina gani?

    Permanent ☐ nyumba ya kudumu

    Semi permanent ☐ nyumba isiyo dumu

13. Do you wish to know your test results for Dengue Virus? /Je ungependa kujua matokeo ya Virusi vya Dengue? Yes/Ndio ☐ No/Hapana ☐

    (If yes, Cellphone number/ Nambari ya simu ____________________________)

Section D: Laboratory assays

Date of sample collection __ dd ___ mm _____ yy

Date and time of arrival to the lab __ dd ___mm_____ yy; Time______

Date of separating into plasma __dd ___mm _______yy

IgG ELISA Positive ☐ negative ☐

Appendix 3: Approvals

ERC Approval

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

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: www.uonbi.ac.ke
Link:www.uonbi.ac.ke/activities/KNHUoN

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/179

24th June 2013

Betty Jepkurui Koech
Dept. of Medical Microbiology
School of Medicine
University of Nairobi.

Dear Betty

RESEARCH PROPOSAL: SEROINCIDENCE AND PREVALENCE OF DENGUE VIRUS IN THE KENYAN POPULATION (P64/02/2013)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 24th June 2013 to 23rd June 2014.

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 severe 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-Ethics & Research Committee 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 **www.uonbi.ac.ke/activities/KNHUoN.**

Yours sincerely

PROF. M. L. CHINDIA
SECRETARY, KNH/UON-ERC

c.c. Prof. A.N. Guantai, Chairperson, KNH/UoN-ERC
Deputy Director CS, KNH
AD, Health Information, KNH
Principal, College of Health Sciences, UoN
Dean, School of Medicine, UoN
Chairman, Dept. of Medical Microbiology, UoN
Supervisors: Prof. O. Anzala, Dr. Julius Oyugi

KNBTs Approval

MINISTRY OF HEALTH

Telephone: 020-2012867
Hotline: +254 716775245
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When replying please quote:
Ref. No. ST/7/1/7 VOL. I

NATIONAL BLOOD TRANSFUSION SERVICE - HQS
LOCATION: KENYATTA NATIONAL HOSPITAL, NPHLS GROUNDS
P.O.BOX 29804-00202
NAIROBI

Date: .....14th..AUGUST, 2013

Betty J. Koech
University of Nairobi

RE: REQUEST TO CONDUCT THE STUDY IN NBTS PREMISES

This is to permit you to carryout the study on dengue fever-in four RBTCs namely Eldoret, Kisumu, Mombasa and Nairobi.

By copy of this letter the Technical Directors of the four RBTCs are requested to discuss with you the modalities and study design on the ground.

Kindly note that HIV results of the donors who enroll in the study will however not be released to you.

You ae expected to discuss the results of your study with NBTS Director before publishing them should you desire to do so.

Thank you.

DR. MARGARET ODUOR
Director, NBTS

Cc: Technical Directors - Nairobi, Eldoret, Mombasa and Kisumu
-National Technical Director

It's safe and it saves.