Assessing the risk of Transmission of Yellow Fever and Dengue viruses by *Aedes* (*Stegomyia*) mosquito populations in Northern Kenya

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Ethics

THIS STUDY HAS BEEN APPROVED BY UNIVERSITY OF PRETORIA RESEARCH ETHICS AND PHD COMMITTEE, PROTOCOL NUMBER **491/2017**.

Declaration

I declare that the thesis, which I hereby submit for the degree of Doctor of Philosophy (Medical Virology) at the University of Pretoria, is my own original work and has not been previously submitted by me for a degree at this or any other tertiary institution.

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List of Abbreviations

AIC: Akaike Information Criterion

aORs: Adjusted odds ratios

BG: Biogent

BI: Breteau Index

BLAST: Basic Local Alignment Search Tool

BSL: Biosafety Level

CDC: Center for Disease Control

CI: Confidence Interval

CI: Container Index

COI: Cytochrome Oxidase I

CO_2: Carbon dioxide

CPE: Cytopathic Effect

CSC: Center for Virus research Scientific Committee

DENV: Dengue virus

DF: Dengue fever

DHF: Dengue Hemorrhagic Fever

DMEM: Dulbecco's Modified eagles Media

DNA: Deoxyribonucleic acid

DnaSP: DNA Sequence Polymorphism

DRC: Democratic Republic of Congo

EID: Emerging Infectious Diseases

F: Filial

FBS: Fetal Bovine Serum

G: Gamma

GIS: Geographic Information System

GLM: General Linear Model

GPS: Global Positioning System

H: Haplotypes

Hd: Haplotype diversity

HI: House Index

I: Invariant

KEMRI: Kenya Medical Research Institute

MEB: Midgut escape barrier

MEGA: Molecular Evolutionary Genetic Analysis

MEM: Minimum Essential Media

MIB: Midgut Infection barrier

ML: Maximum Likelihood

NHP: Non-human primate

NGS: Next Generation Sequencing

P: Calculated probability

PCR: Polymerase Chain Reaction

PFU: Plaque Forming Units

Pi: Nucleotide diversity

PRNT: Plaque Reduction Neutralization Test

RCF: Relative Centrifugal Force

RH: Relative Humidity

RNA: Ribonucleic Acid

RVF: Rift Valley Fever

SERU: Scientific and Ethics Review Unit

SD: Standard Deviation

TN: Tamura-Nei

TOT: Transovarial Transmission

VC: Vector Competence

WHO: World Health Organization

WNV: West Nile virus

YF: Yellow Fever

YFV: Yellow fever virus

ZIKV: Zika virus

Presentations and Publications related to this work

Presentations

October 2018: Presented on "Serological evidence of *Flavivirus* circulation in human populations in Northern Kenya: An assessment of disease risk 2016-2018" at the 67th American Society of Tropical Medicine and Hygiene (ASTMH) annual meeting held on October 28-November 1, 2018 at the Sheraton New Orleans and New Orleans Marriott in New Orleans, Louisiana USA.

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Manuscripts

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Summary

East Africa has been experiencing an increase in the occurrence of emerging infectious diseases such as yellow fever (YF) and dengue (DEN). Increasing frequency of YF activity in East Africa constitutes a re-emergence that was not detected for over 40 years. Additionally, DEN outbreaks have also increased in frequency and continue to be detected in Kenya and in neighboring countries like Tanzania, Somalia, Djibouti, Eritrea and South Sudan. The renewed vigor of YF and dengue fever (DF) re-emergence in East Africa presents a new challenge to public health in spite of the availability of a safe and effective vaccine for YF. However, there is need to understand the potential for YF and DEN transmission along the border areas of Kenya, because Kenya is classified among countries with medium to high risk for YF transmission. This classification was mainly based on historical data, proximity to countries reporting recent YF outbreaks, the presence of non-human primates known reservoirs for these viruses, unrestricted human movement and presence of potential vector mosquito species. Both YF and DEN share a similar niche in the ecosystem and are associated with *Aedes* mosquito species of the subgenus *Stegomyia*. While the factors leading to the re-emergence of these diseases are poorly understood, a better epidemiologic understanding relating to disease ecology including presence of potential vectors, their host blood feeding preferences, the vector competence in transmission of these viruses and evidence of virus circulation in human population, will guide assessment of disease risk in the target areas and help to prevent or mitigate severe outbreaks in this region. We hyothesised that the key *Stegomyia* species are neither abundant nor diverse, and are not anthropophilic; therefore, there is low human exposure to YF and DEN infections, and no variation in the genetics and competence of the key vectors in West Pokot and Turkana counties in Northern Kenya.

With increasing YF and DEN outbreaks being reported regionally (Uganda, South Sudan and Ethiopia), Chapter 2 shows the findings on the vector species occurrence, composition and blood feeding patterns of the *Stegomyia* species, in West Pokot County which borders Uganda. Knowledge on the distribution of potential vectors involved, including the critical aspects of their ecology can reveal information about potential reservoirs of disease and risk of transmission to susceptible populations. Adult mosquitoes were sampled using CO_2-baited BG Sentinel traps at three time points during the rainy season (short and long rain seasons) from West Pokot County. Abundance patterns (response variable) with emphasis on *Aedes aegypti* and other *Stegomyia* species, and diversity patterns were compared using generalized linear models (GLM) with negative binomial error structure across the sampling periods (predictor variables). Blood fed specimens were processed for host blood meal sources by Polymerase Chain Reaction (PCR) targeting 12S rRNA gene followed by sequencing. The findings showed *Ae. aegypti* being the most prevalent among *Stegomyia* species, followed by *Ae. vittatus* and *Ae. metallicus*, which are known potential vectors of YF and DEN viruses. There was a significant variation in mosquito diversity and species richness across the sampling periods, which correlated with the period of highest rainfall. The *Stegomyia* species exhibited zoophagic tendency with the mosquitoes mainly feeding on rock hyraxes, goats, cattle and sheep, and few fed on humans.

In Chapter 3, this study sought to determine the seroprevalence of yellow fever, dengue, West Nile and Zika viruses among the human populations in this region. An understanding of the presence and spread of YF, DEN and other closely related Flaviviral diseases in West Pokot and Turkana counties in Northern Kenya will guide in control measures and advice on the need for YF vaccination strategies. These two counties are located in areas that border countries that have had and recently reported outbreaks of these *Flaviviruses*. Flavivirus antibodies have been

reported to show cross-reactivity with other *Flaviviruses*, in light of this, West Nile and Zika viruses, which have previously been reported to circulate in the border countries, were included in panel for screening. Serum samples were collected from asymptomatic human population in the region and Plaque Reduction Neutralisation Test (PRNT), the most virus-specific serologic test that measures the biological parameter of *in vitro* virus neutralization, was used to determine the level of human exposure to these *Flaviviruses*. Seroprevalence was compared by county, site and important human demographic characteristics. Adjusted odds ratios (aOR) were estimated using Firth logistic regression model. The findings demonstrated human exposure to infections with *Flaviviruses* in the two counties in Northern Kenya. While the observed YF prevalence in Turkana and West Pokot counties may imply virus activity, this could also be as a result of vaccination following the YF outbreak in the border countries, Ethiopia, South Sudan and Uganda.

The epidemiologic trends observed in the previous chapters of this study could reflect differences in the population structure of the local vector. In view of this, Chapter 4 report's on the test of the hypothesis that, population differences of the YF and DEN vector, *Ae. aegypti*, exists between the two areas. The main objective was to compare the genetic diversity and distribution of the forms of *Ae. aegypti* from both areas and assess the vector competence of the species for dengue-2 virus. Mitochondrial sequence data which has been valuable in characterising vector genetics was employed to determine the genetic variability of *Ae. aegypti* collected from West Pokot and Turkana counties. The barcode region of the Cytochrome Oxidase subunit 1 (*COI*) gene was amplified using published *COI* primers. Additionally, vector competence experiments were performed to determine the susceptibility of *Ae. aegypti* from this region, for DENV-2. The Neighbor joining phylogeny based on *COI* gene sequences revealed

the presence of two lineages. In one, all samples from Turkana and a proportion from West Pokot formed a lineage, clustered with the sylvatic *Ae. aegypti formosus*. The second lineage exclusively had samples from West Pokot County, clustered with the domestic form of *Ae. aegypti*. Interestingly, *Ae. aegypti* population from West Pokot were more susceptible to dengue-2 virus compared to the mosquito population from Turkana County, though there was no disseminated infection.

In conclusion, the burden of YF and dengue in East Africa represents a growing challenge to public health officials and policymakers. The success in tackling this growing threat is, in part, dependent on strengthening the evidence base on which control planning decisions and their impact are evaluated. Generally, our data does not support the hypothesis. The study provides a useful baseline and first report on the mosquito fauna inhabiting the ecology of this study area in Kenya. It also shows the evidence of circulation of *Flaviviruses* of medical importance in the human population. The variable human exposure risk observed between the areas could be related to the differences in climate and geography of the two areas. The study confirms the presence of both subspecies of *Ae. aegypti* in the Northern region and the difference in their susceptibility to dengue-2 virus varied significantly. This study certainly provides useful information and knowledge needed to make a comprehensive risk assessment package; including understanding potential for virus re-emergence and spread, virus circulation and the populations at risk in this border region. However, to conclusively identify the genetic basis of the mosquito adaptation, it will be necessary to include more mosquito populations, to allow for more powerful tests of selection and ultimately link these differences to phenotypic changes. More bionomic and active surveillance studies through one health approach, to integrate the myriad of

factors involved in modulating human exposure risk and mosquitoes present in this area will be invaluable.

Chapter 1

LITERATURE OVERVIEW

1.1 Introduction

Arthropod-borne viruses (arboviruses) are predominately RNA viruses, propagated through biological transmission between susceptible vertebrate hosts by hematophagous arthropod vectors, mainly mosquitoes, biting midges, sandflies and ticks (Hollidge et al., 2010). Arboviruses of medical relevance are mainly classified into seven families; *Togaviridae, Phenuiviridae, Peribunyaviridae, Flaviviridae, Rhabdoviridae, Orthomyxoviridae* and *Reoviridae* (ICTV, 2018; Calisher et al., 2019). Arboviruses are increasingly becoming a threat to human and livestock health with rising frequency of outbreaks around the globe, with a number of the responsible agents having their origins in Africa (Gubler, 2002; Weaver and Reisen, 2010). These viruses occur in sylvatic, rural, urban and peri-urban environment where they circulate among wild animals and cause disease after spillover transmission to humans and/or domestic animals that are incidental or dead-end hosts causing considerable morbidity and mortality (Gubler, 2002).

Being so close to the equator, the climatic conditions prevailing in much of East Africa, permits seasonal circulation of viruses. In hotter and humid regions, high abundance of vectors may sustain virus transmission occasionally. Seasonality in the tropics influences mosquito populations, as the duration of wet and dry seasons affects larval development and adult abundance (Agha et al., 2017b; Chepkorir et al., 2018). During the wet seasons, the rains create

more breeding habitats for mosquitoes, and the elevated humidity levels extend the lifespan of adults, thus prolonging disease transmission rates (Patz et al., 2000). This may cause outbreaks when transmission occurs in naive human or animal population where the virus has not occurred or is re-emerging causing public health, economic distress and anxiety (Weaver and Barrett, 2004). Other factors including human activities and viral genetics contribute to arbovirus emergence and re-emergence (Patz et al., 2000).

In Africa, arbovirus outbreaks are occurring with increasing frequency and geographic spread, with diseases such as Rift Valley fever virus (RVFV), chikungunya virus (CHIKV), dengue virus (DENV) and yellow fever virus (YFV) being reported in Kenya, Uganda, Tanzania, Somalia, Ethiopia, Angola and Democratic Republic of Congo (DRC) (Sang et al., 2017; Konongoi et al., 2018; Mboera et al., 2016; Wamala et al., 2012; Himeidan et al., 2014; WHO, 2016; Lilay et al., 2017; Kraemer et al., 2017). The potential for these viruses to spread beyond their emergence zones exists as has been demonstrated by the recent spread of DENV, CHIKV and Zika virus (ZIKV) from the African continent to Europe and the Americas where they have been causing outbreaks among immunologically naive populations with unprecedented public health consequences (Moi et al., 2010; Shiferaw et al., 2015).

Despite the public health impact and epidemic potential of arboviruses, there are no vaccines or specific therapeutic treatment available for prevention and/or management of human or animal infections for most of them (Gubler, 2002). Thus, knowing their mode of transmission and the ecology of associated vectors provides much needed information useful for targeted prevention and/or control of outbreaks and may be helpful in delineating distribution and risk foci of arboviruses.

Surveillance for arbovirus vectors is a critical component of risk assessment for their transmission and outbreak occurrence. This requires knowledge on the disease and vectors involved including their potential to sustain these viruses, which are the critical aspects of the vector ecology. For example, knowledge about their host blood feeding preference can reveal information about potential reservoirs of diseases transmitted by these vectors. Again, the chemical basis for attraction to preferred hosts can be exploited in the development of odor baited traps for improved surveillance of the target vectors as attempted for *Aedes* mosquito vectors (Tchouassi et al., 2019). Moreover, attempts have been made to develop a more sensitive and an effective trapping system for vectors of YFV and DENV (Owino et al., 2015) of critical need to maximize viral detection from trapped vectors for accurate risk assessment and outbreak prediction. In Kenya, most surveillance studies have focused on areas like Northeastern, coastal and urbanised areas (Ochieng et al., 2013; Agha et al., 2017a; Agha et al., 2019), yet poorly understood in dry ecologies or less urbanised settings of West Pokot and Turkana counties in the Northern region.

1.2 The family *Flaviviridae*

The family *Flaviviridae* comprises the genera *Flavivirus*, *Pestivirus*, *Pegivirus* and *Hepacivirus*. The genus *Flavivirus* consists of 53 virus species, which make up a large portion of the *Flaviviridae* family (Simmonds et al., 2017). Arboviruses within the genus *Flavivirus* are transmitted by a variety of mosquito species as well as ixodid and argasid ticks (Lawrie et al., 2004; Lwande et al., 2013). Here, the focus is on four medically important flaviviruses that have previously been reported to be circulating in East Africa: YFV, DENV, ZIKV and West Nile virus (WNV). *Flavivirus* antibodies have been reported to show cross-reactivity with other

closely related *Flaviviruses* and therefore, the need to focus on the four to rule out cross-reactivity. The geographic distribution of viruses in this family is very broad, and consistent with other arboviruses, the distribution of each virus mirrors that of its vector (Huang et al., 2014).

It has been estimated that over half of the global population is at risk for infection with one of four dengue virus serotypes (DENV-1, -2, -3, and -4) (Gubler, 2004), and YFV, DENV, WNV collectively cause millions of infections and tens of thousands of deaths each year (Kuno and Chang, 2005). Additionally, the re-emergence of ZIKV from the original African strain causing a mild febrile illness with rash in East Africa (Dick et al., 1952) to the Asian strain associated with life threatening conditions and/or severe sequelae (Higgs, 2016) informs the need for constant monitoring of the circulation of these viruses. Syndromes following human infection with flaviviruses range from clinically inapparent asymptomatic infections to severe, and sometimes, fatal disease, including hemorrhagic manifestations of severe YFV and DENV infection, microcephaly and other congenital malformations with ZIKV infection, and encephalitis caused by infection with WNV. Whereas humans are dead-end hosts for many arboviruses, including WNV (Bowen and Nemeth, 2007), they play a large role in the transmission cycles of YFV, ZIKV and DENV (Gubler, 2004; Barret and Higgs, 2007; Higgs, 2016).

1.3 Genomes organisation and Clinical presentation of Yellow fever and Dengue

Dengue virus and YFV are closely related and remarkably similar. The flavivirus genome consists of a single-stranded, positive sense RNA which is approximately 11 kb in length with a single long open reading frame (Ciota and Kramer, 2010). The flavivirus genome encodes a polyprotein that is cleaved to produce three structural proteins (Capsid (C), Membrane Protein

(PrM) and Envelope (E)) and seven non-structural (NS) proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5). The 5' end is capped and the 3' end lacks a poly (A) tail (Kümmerer et al., 2018). Virus entry occurs by receptor-mediated endocytosis, the acidic pH in the endosome triggers structural alterations in the E protein that lead to the fusion of the viral membrane with the endosomal membrane. Genome replication occurs in the cytoplasm in association with modified cellular membranes. Viral particles are transported in cytoplasmic vesicles through the secretory pathway before they are released by exocytosis (Bollati et al., 2010). It is known that the purified RNA of *Flaviviruses* is infectious and allows initiation of a complete viral life cycle by transfecting the genomic RNA into susceptible cells. All *Flaviviruses* are relatively unstable in the environment and thus easily inactivated by heat and by common disinfectants.

In humans, infections with YFV cause a broad spectrum of disease, ranging from mild symptoms to severe acute hemorrhagic fever with high case fatality rates. Clinical symptoms of YF typically appear 3-6 days after an infective mosquito bite, but only in about 15% of those infected, with the majority only experiencing a mild disease. About 20–60% of cases develop severe acute illness with fever, nausea, vomiting, epigastric pain, hepatitis with jaundice, renal failure, hemorrhage, shock and death (Monath & Vasconcelos, 2015). The first phase manifests with sudden onset of fever, headache, muscle pain, backache, general weakness, failure of pulse to rise with temperature (Faget's sign), red eyes (injected conjunctiva), nausea and vomiting during which patients have viremia, and can infect mosquitoes. This is followed by a short period of remission for about 24 hours. The second phase (toxic phase) begins manifesting with high fever, vomiting, epigastric pains, jaundice, hemorrhagic diathesis (hematemesis) and coma, and could result in death (Monath, 1989).

Dengue illnesses are caused by any of the four serologically related, but antigenically distinct viruses designated as DENV-1, DENV-2, DENV-3, DENV-4 (Gubler, 1997). Infection with any one of these serotypes causes undifferentiated illness, classic dengue fever (DF); however, a few cases develop severe life threatening dengue hemorrhagic fever (DHF) and dengue shock syndrome (Gubler & Clark, 1995). Undifferentiated dengue, the most common syndrome, occurs when DENV infection is asymptomatic or mildly symptomatic (Rigau-Perez et al., 1998). Although classical dengue is not usually fatal it has very high morbidity, its alternate name is break-bone fever for the severe joint pain during infection. Disease is most common in infants, young children, and adults. Classical dengue manifests itself with a mild to high fever, red rash, debilitating headaches, muscle and joint pain lasting 2-7 days (George & Lum, 1997). Dengue shock syndrome results when capillaries leak. This leads to edema (swelling of tissue because of fluid escaping the circulatory system), abdominal pain (as a result of the edema) and hypotension (low blood pressure resulting from a drop in total blood volume from fluid loss). Oxygen and nutrients stop reaching the body tissues because of inadequate circulation of the blood as a result of these symptoms and this can lead to shock and death. Dengue hemorrhagic fever occurs when normal blood coagulation is disrupted by infection. Fever, emesis, and bleeding are all symptoms common to dengue hemorrhagic fever. The results of internal hemorrhage can be seen in the form of tiny red spots (petechiae) or sometimes patches under the skin as well as bloody stool and bleeding from the gums and nose. Additionally, the cause of the more severe dengue syndromes may be antibody mediated enhancement, after reinfection with another serotype. Mortality from these complications can be up to 14% without proper care (Deubel & Murgue, 2001).

1.4 Epidemiology of Yellow fever and Dengue

1.4.1 Global burden of Yellow fever and dengue

Yellow fever (YF) is an acute, often fatal infectious disease occurring in sub-Saharan Africa and tropical America. Yellow fever virus, that causes YF, was first isolated in West Africa in 1927 (Barrett & Higgs, 2007). The number of cases has increased over the past two decades (WHO, 2011), perhaps because of decreased population immunity to this infection, deforestation, urbanisation, population migration from rural to urban areas and vice versa and the appearance of new vectors. There is an estimated annual YF incidence of 200,000 cases, and 30,000 deaths, mostly in sub-Saharan Africa in which 33 countries and over 500 million people are at risk (WHO, 2014; Garske et al., 2014). It is therefore, crucial to implement vector control operations in order to limit human–vector contact (Farnon et al., 2010). In Africa, YF is endemic in 33 countries and continues to cause severe morbidity and mortality (WHO, 2014) despite the availability of an effective vaccine.

Dengue is one of the notable viral infections, the global epidemiology of which has changed dramatically in the past 50 years but especially during the last 20 years in the tropical regions of the world (Gubler, 1997). Dengue is a major public health problem worldwide, especially in the tropical and subtropical areas with around 2.5 billion people living in areas at risk (WHO, 2007). In Africa, the epidemiology and public health effect of dengue remains largely uncharacterized, and the available information and sequence data are fragmented (Amarasinghe *et al.,* 2011). When the distribution of the principal vector, *Aedes aegypti* is combined with rapid population growth, unplanned urbanisation, and increased international travel, extensive transmission of DENV is likely in Africa (Gubler, 2004). Over the past 5 decades, cases of epidemic or sporadic dengue have been reported in many countries in sub-Saharan Africa (WHO, 2009). However,

when compared with the Asia–Pacific and Americas–Caribbean regions, the epidemiology and burden of dengue in Africa has not been defined (Sang, 2007).

1.4.2 Yellow Fever and dengue in East Africa

A resurgence of YF incidence (Ellis & Barrett, 2008), over recent decades, has reinvigorated public health and research interest; however, there remains a clear need for improved surveillance, a re-evaluation of risk assessment indicators and an expanded immunisation programme (Ellis & Barrett, 2008; Gubler, 2004). East Africa is particularly vulnerable to the emergence of these diseases as evidenced by the largest epidemic of YF reported worldwide (Ethiopia 1960 –1962, 2013) and outbreaks in Kenya (1992 –1993), Sudan (2003, 2005, 2010) and Uganda (2011, 2016, 2019, 2020) (Onyango et al., 2004; WHO, 2005; WHO, 2016; Reiter et al., 1998; Sanders et al., 1998; Lilay et al., 2017). An accurate risk assessment of YF emergence requires a thorough understanding of the disease ecology and epidemiology at both local and regional scales. There is only limited information on the situation in East Africa (Ellis & Barrett, 2008), in terms of the ecology and the availability of potentially competent vectors. Unfortunately, this is due to the dynamic nature of the disease, unpredictable focal outbreak periodicity and a paucity of recent local research data in East Africa. There is also the element of poor understanding and misdiagnosis especially with diseases like malaria because of similarity in clinical presentation especially in the early stages of infections (Sang, 2007).

Dengue outbreaks have also increased in frequency in the region with reports in Tanzania, Somalia, Djibouti, Eritrea and Sudan (Sang, 2007; AFENET, 2013; Mboera et al., 2016). The greatest threat of YF and dengue to East Africa is the potential emergence of the disease from sylvan areas, following proximal epizootic activity, and subsequent introduction into urban areas

with dense populations of susceptible hosts and domestic vectors (Gubler, 2004). Emergence of urban YF could be mediated by the adaptation of sylvatic YFV virus to domestic vectors as has been previously suggested for dengue that is also re-emerging with renewed vigor in East Africa. The adaptation of sylvatic vectors to rural or urban settings could similarly lead to emergence of these viruses in rural and urban areas. Generally, both YF and DEN risk factors fall into three main categories: human, mosquito, and non-human primate (NHP) host, influenced by the climate and ecological environment (Briand et al., 2009). The scenario underscores the importance of understanding the types of vectors present in these ecological interfaces and evaluating the vector competence of the populations of potential vectors.

1.4.3 Yellow Fever and dengue in Kenya

Outbreaks of YF were last reported in Kenya in 1992-1995 (Reiter et al., 1998; Sanders et al., 1998). Mass vaccination of locals following the outbreak was carried out and there have been no reported cases since then. Despite this, Kenya is still being classified in the medium to high risk list of countries for YF. With the recent outbreak of YF in neighboring Uganda (WHO, 2011; WHO, 2016; WHO, 2020), the risk of spill over to Kenya remains a possibility; additionally, salient local transmission in previous endemic areas is a possibility following re-introduction from non-human primate reservoirs. As such, research to assess risk of the disease along border areas of Kenya remains a priority. This would generate crucial data that can contribute to existing information gathering by the Kenyan government to support a case to have Kenya reclassified from the high-risk status to a low-risk country.

In 1982, an outbreak of dengue fever caused by dengue-2 virus (DEN-2) was first reported in the Kenyan coastal towns of Malindi and Kilifi (Johnson et al., 1982); clinical presentation was

consistent with classical dengue fever, with no severe dengue reported. The 1982 outbreak in Kenya is believed to have spread from the Seychelles outbreak that occurred between 1977 and 1979 (Metselaar et al., 1980). Since then there have been sporadic cases of dengue reported in Kenya and a serology survey carried out in 2005 revealed the occurrence of dengue transmission in coastal and inland parts of Kenya (Mease et al., 2011). In 2012, the second dengue outbreak in Kenya occurred in Mandera in the North Eastern Kenya. Subsequently between 2013 and 2014, another dengue outbreak occurred in Mombasa (Coastal Kenya), where Den-1, -2 and -3 were detected (Ellis et al., 2015; Konongoi et al., 2016). The disease is currently endemic in parts of the Coastal Kenya with periodic outbreaks being reported in the recent times.

1.5 Transmission cycles and Vectors for Yellow fever and Dengue

1.5.1 Transmission cycles

DENV and YFV are primarily transmitted by mosquitoes of the genus *Aedes* and subgenus *Stegomyia* (Huang, 2004). While YFV originated in Africa it is uncertain where DENV originated from. Both viruses originated in sylvatic cycles, are maintained in non-human primates and forest-dwelling *Aedes* mosquitoes, and have a history of successful emergence into sustained transmission among humans by *Aedes aegypti* (Hanley et al., 2013). YFV circulates in three ecologically distinct transmission cycles, each with its own suite of vectors (Barrett & Higgs, 2007; Monath, 1989). Previously in South America, YFV only existed in urban cycle between humans and *Aedes aegypti,* but recent studies have shown that YF is endemic in the region, with sylvatic transmission reported since 2016 (Rezende et al., 2018; Silva et al., 2020). In Africa, three transmission cycles have been recognised: the jungle cycle between monkeys and the canopy-dwelling *Ae. africanus,* the intermediate cycle or zone of emergence, where the

virus circulates between monkeys and/or humans and mosquitoes such as *Ae. africanus*, *Ae. furcifer–taylori*, *Ae. luteocephalus*, and members of the *Ae. simpsoni* complex, and an urban cycle between humans and *Aedes aegypti* (Hanley et al., 2013).

Dengue virus occurs in sylvatic cycles in Africa. The sylvatic vectors in Africa include *Aedes (Stegomyia) africanus*, *Ae. (Stegomyia) luteocephalus*, *Ae. (Stegomyia) opok*, *Ae. (Diceromyia) taylori*, and *Ae. (Diceromyia) furcifer* (Diallo et al., 2003). These sylvatic cycles, are believed to represent the ancestral DENV cycles from which epidemic/endemic strains of DENV-1 to -4 evolved. Although humans occasionally become infected with sylvatic DENV in Africa and perhaps in Asia, they are tangential to the maintenance cycle, which involves sylvatic *Aedes* species mosquito vectors and non-human primates as reservoir hosts (Figure 1).

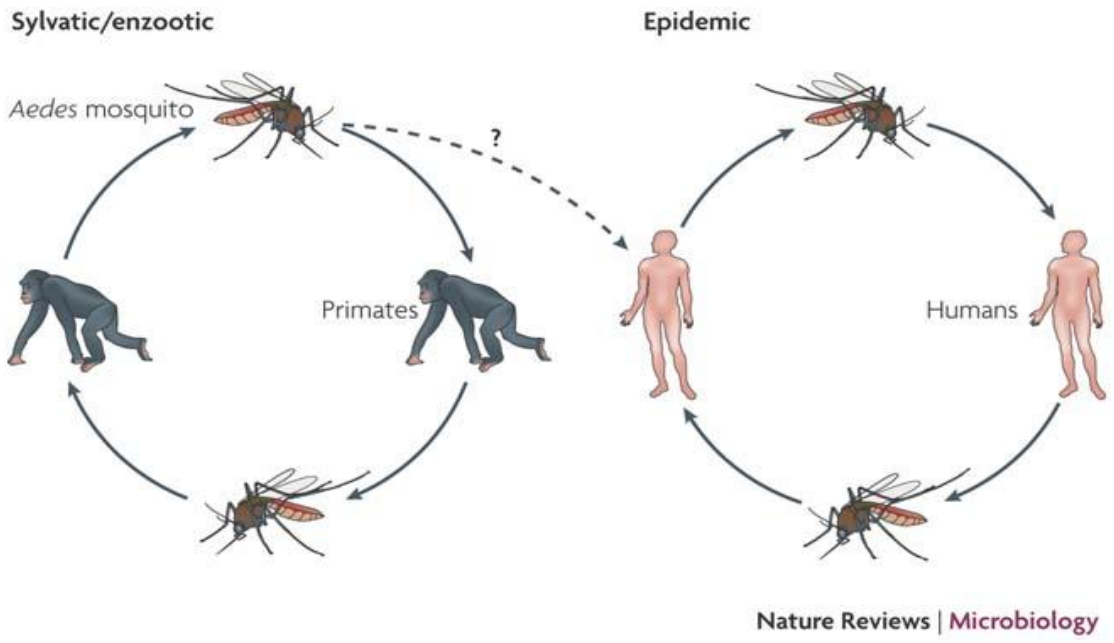

Figure 1: Yellow fever and Dengue transmission cycles (Source: Nature reviews Microbiology 2007; 5: 518 – 528)

The ancestral sylvatic cycles of both YFV and DENV are constrained to a subset of the geographic regions where potential hosts and vectors occur. This cycle of alternating infection of vertebrates and arthropods imposes substantial constraints on arbovirus evolution (Weaver, 2006). Despite the current restricted geographical distribution of YFV, there is a risk of expansion through a viremic traveler, who may come in contact with local competent YFV vectors in a novel location (van den Hurk et al., 2011).

1.5.2 Vectors of Yellow fever and Dengue viruses

Several vectors have been implicated in the transmission or maintenance of YF through virus isolation, while many others have been suspected because of their population dynamics (vector abundance, blood feeding preference and climatic conditions), behavior and/or ability to transmit YFV under laboratory conditions. Individual species may be implicated as vectors when several factors are considered including their geographic distribution, the total number and frequency of virus isolations from the species and experimental determination of either vector competence and/or transovarial transmission (TOT) (Ellis and Barrett, 2008). The role of *Ae. africanus* in enzootic jungle transmission cycles was originally described in Uganda in 1948, and has since proven to be an important YF vector in many forested areas throughout Africa (Smithburn et al., 1949).

In Central Africa *Ae. (Stegoymia) opok* may be found in higher abundance than *Ae. africanus* and considered a more important local vector for YF (Hervé et al., 1977). *Aedes africanus* is the most important vector of YF in forested areas of Africa and has produced the highest number of YF isolations in the most number of countries (Ellis and Barrett, 2008). These vectors are

predominantly involved in monkey-to-human transmission of YFV in forested areas. This contrasts markedly from the epidemiology associated with the main peridomestic/domestic YFV vector in East Africa, *Ae. bromeliae* (Huang, 1986). *Ae. bromeliae* has proven epidemic transmission capacity and was the principal vector implicated during the largest recorded epidemic of YF worldwide, in Ethiopia from 1960 to 1962 (Haddow, 1965). This species is also considered an important bridge vector in areas of Uganda where it feeds almost exclusively on man and is found prolifically in banana plantations that may border forested areas with proximal YF activity (Haddow, 1969).

Outbreaks of YF in East and Central Africa so far, have been associated with sylvatic mosquito vectors including members of the *Ae. simpsoni* species complex (*Ae. simpsoni*, *Ae. bromeliae* and *Ae. lilii*). In East Africa, *Ae. aegypti* exists in human-biting (anthropophilic) and non-human-biting (sylvatic) populations, but YF outbreaks have not been reported even in areas where *Ae. aegypti* readily bite humans such as along the coast of Kenya (Trpis & Hausermann, 1975). Urban epidemics of YFV, vectored by domestic *Ae. aegypti* species had never occurred in East and Central Africa, until recently during the 2016 YF outbreak in Angola and DRC (Kraemer et al., 2017). This scenario may have changed following the outbreak and risk of urban YF in these regions should be cause of concern. The first documented YF outbreak in Kenya occurred in the year 1992–1993 in the southern part of the Kerio Valley, Elgeyo Marakwet county and in the adjacent parts of Baringo county (Sanders et al., 1998). During the outbreak, *Ae. aegypti* was not incriminated as the vector; but *Ae. africanus* and *Ae. keniensis* through virus isolation (Reiter et al., 1998). Additionally, the possibility of *Ae. bromeliae*, *Ae. luteocephalus*, *Ae. metallicus* and *Ae. vittatus* being involved in transmission may not be ruled out, since they were abundant at the initial stages of the outbreak. The entomologic evidence and the observed YF cases, confirmed

that this was a sylvatic outbreak (Reiter et al., 1998) and currently several countries in Africa are at risk of YF transmission (Figure 2).

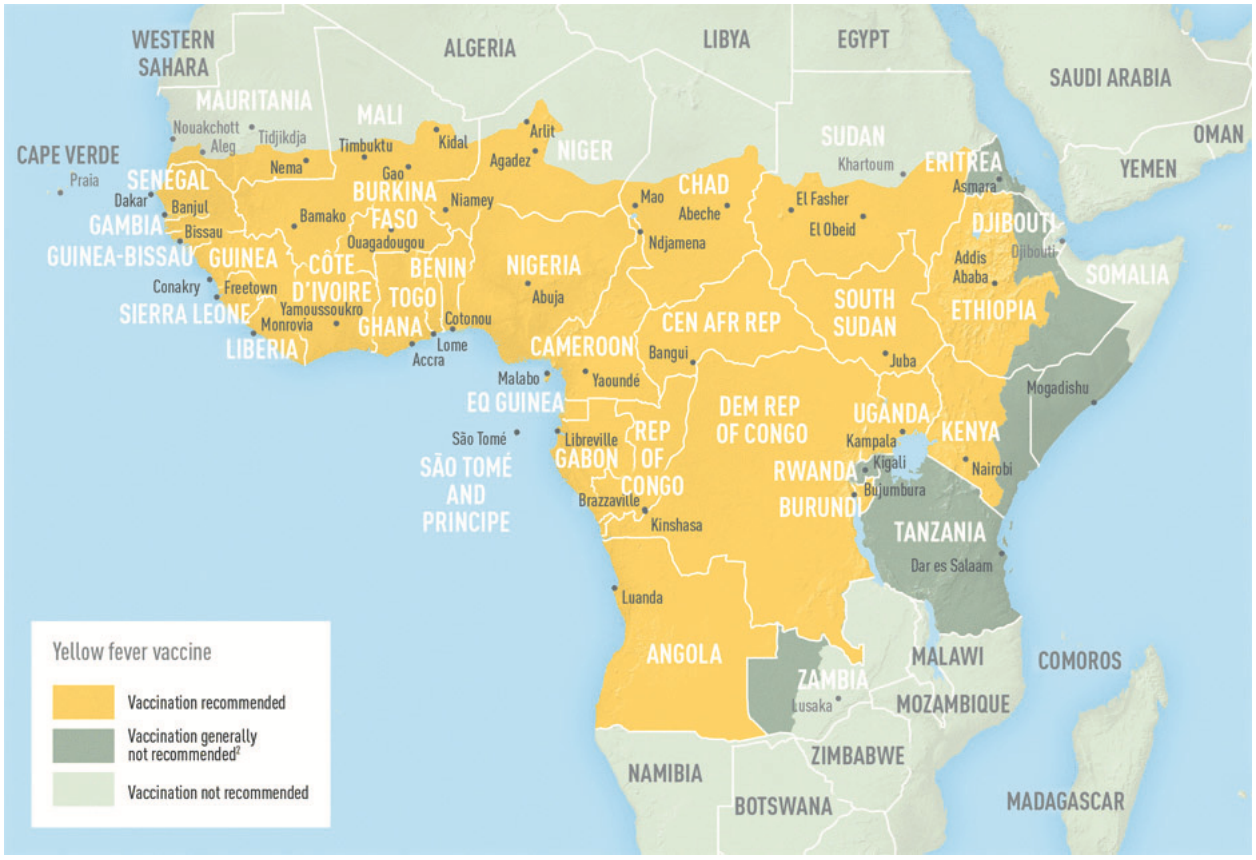

Figure 2: Map of Africa showing countries at risk of yellow fever transmission. Map created by the Informal WHO Working Group on the Geographic Risk of Yellow Fever (January 2017).

DENV is principally transmitted by *Ae. aegypti*, which originated in Sub-saharan Africa and spread to other countries in Africa, and other tropical countries in the 17th and 18th centuries (Halstead, 2008). Several other *Aedes* species mosquitoes, including *Ae. albopictus*, *Ae. africanus*, and *Ae. luteocephalus*, are found in Africa and are potential DENV vectors. Urbanisation is a major factor in facilitating the increase of *Aedes (Stegomyia)* mosquito species populations (Gubler & Clark, 1995). Accumulation of non-biodegradable, human-made

containers used to store water in and around living areas (Monath, 1994) and discarded containers due poor waste disposal habits, has provided the aquatic environment to which these mosquitoes are best adapted. Since the 1950s, a 3-fold increase in urban human population density has occurred in Africa, and with these demographic changes and subsequent increases in *Aedes* mosquito populations, increased DENV transmission is likely to occur in Africa (Appawu et al., 2006).

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). In the contrary, during the dengue fever outbreak caused by dengue 2 virus in 1976-1977 in the Seychelles, *Ae. albopictus* was considered the sole vector. This was mainly because it was abundant in human habitations and dengue 2 virus was isolated from pools of *Ae. albopictus* during the outbreak (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 (Diallo et al., 2008). Thus, appropriate ecologic studies are needed in Africa to determine the relative roles of each species in transmission of DENV.

1.6 Mosquito host blood feeding preferences

Mosquito biting frequency and how bites are distributed among humans and other animals can have significant epidemiologic impact. Blood meal from human or animal hosts predispose mosquitoes like other disease vectors to become infected with pathogens if the host is infected. An improved understanding of mosquito host blood feeding preferences would refine knowledge

of the entomological processes supporting pathogen transmission. It could also reveal targets for minimizing risk and breaking pathogen transmission cycles (Harrington et al., 2014). Many mosquitoes express an opportunistic trait of host choice, feeding on a wide range of animals while others are specialists feeding on only a narrow range if not specific hosts. Host preference of mosquitoes is affected by extrinsic and intrinsic determinants, of which genetics is an important component. Many species express inherent traits in host preference (birds or mammals), but this preference is readily overruled by physiological factors (hunger) and physical abundance of available hosts. Therefore, knowledge on feeding preference can reveal important information about potential pathogen reservoirs important in the epidemiology of a disease.

Studies on *Aedes aegypti* mosquito species guided by blood meal analysis of trapped mosquitoes, in urban Kenya, have shown that apart from feeding on human, other suitable domestic animals fed on by the mosquitoes were cow, dog, goat and cats (Agha et al., 2019). Based on this finding, other studies have explored the use of crude skin odors trapped on cotton material from humans and non-human primates as lures in the sylvatic and domestic environments (Tchouassi et al., 2019). We believe that this exciting innovative approach should allow us to quickly and reliably identify suitable hosts at the proposed study sites to aid in the development of an effective surveillance tool for vectors of YF and dengue viruses. Additionally, blood feeding on humans is a crucial component of vectorial capacity, which is a measure of the transmission potential of a given vector. Higher human blood feeding has been identified as an important driver of dengue emergence (Agha et al., 2019), thus underscores the importance of *Ae*. *aegypti* feeding behavior in the emergence of disease causing pathogens.

1.7 Vector competence of mosquitoes for Viruses

Vector competence (VC) is the intrinsic permissiveness of an arthropod vector for infection, replication and transmission of a virus (Turell et al., 1984). The competence of a vector is mainly mediated by the presence of several genetically determined barriers to viral transmission, these are; midgut infection barrier (MIB) that prevents invasion and replication of the viruses in the midgut of the mosquito and a midgut escape barrier (MEB) that prevents dissemination of the virus from the midgut to other mosquito body tissues (Black & Bennett, 2002). These barriers are major determinants of vector competence to viruses during experimental infections (Bennett et al., 2002). Their variation in prevalence in natural populations, lead to large intraspecific variation of the mosquito species vector competence and may determine the epidemiology of viruses (Bosio et al., 1998; Black & Bennett, 2002).

When a mosquito ingests a viremic bloodmeal, the first step of infection is on the midgut epithelial cells. Passage of the MIB requires the virus to attach, penetrate and replicate in the midgut epithelial cells. Following the successful establishment of infection in the midgut epithelial cells, the infectious virions generated must overcome the MEB which involves passing through the basal lamina and the haemocele in order to establish infection in secondary target organs (Black & Bennett, 2002).

Studies on the flaviviral vector competence of *Aedes* mosquitoes have suggested that the midgut infection barrier is the major determinant of transmission (Bosio et al., 1998). Vector competence studies for YF virus have been carried out in some parts of Kenya. According to a study by Ellis (Ellis et al., 2012) on the competence of *Ae. aegypti* and *Ae. (Stegomyia) simpsoni*, a significant difference in YF virus dissemination rates were observed between populations of

the species collected from four sites tested; Rabai, Kerio Valley and Kakamega and within Nairobi. A dengue vector competence study by Chepkorir (Chepkorir et al., 2014) on mosquito populations from two different geographical locations in Kenya, showed that *Ae. aegypti* population from Kilifi was deemed to be more competent to transmit dengue-2 virus, due to its ability to disseminate the virus, compared to that from Nairobi. This parameter, therefore, gives vector competence its epidemiologic importance in risk assessment and targeting mosquito species of ecologic relevance.

1.8 Seroprevalence of Yellow fever and Dengue in Kenya

Arthropod-borne viral outbreaks have occurred sporadically across regions of Kenya, but few surveillance efforts have taken place to determine the level of human exposure. Limited surveillance efforts in the years between outbreaks suggest low-level endemic transmission of YF, dengue, and other Flaviviral diseases like West Nile and Zika, even outside initial geographic outbreak boundaries (Ochieng et al., 2015, Mease et al., 2011, Sutherland et al., 2011, Geser et al., 1970). Many more diseases of arboviral origin have likely occurred, but have gone unrecognised, especially in more remote locations like in Northern Kenya. Medically important arthropod vectors flourish in Northern parts of Kenya (Lutomiah et al., 2013, Ochieng et al., 2013, Chepkorir et al., 2018). Given the vectorial capacity for arboviral transmission in the region and the history of confirmed arboviral outbreaks, serosurvey is an important component for understanding the circulating, but perhaps rarely diagnosed, human arboviral transmission prevalence (Geser et al., 1970). Currently, there is limited serosurvey data concerning YF and dengue infection in Northern Kenya or any other human *Flavivirus* infections that are most prevalent. In the 1970s, following the largest outbreak of YF in the Omo River Valley in

Ethiopia in 1968 (Serie et al., 1968a; Serie et al., 1968b), studies were conducted to determine the extent of transmission beyond the Ethiopian border into Kenya. Up to 22% YF seroprevalence was detected in Marsabit in Northern Kenya (Henderson et al., 1970), and between 7% and 14.5% YF seroprevalence in Lodwar and Lokitoung (Henderson et al., 1968). Although emergent disease outbreaks in Kenya have confirmed the presence of a number of arboviral diseases within the human populations, much still yet to be known regarding the true prevalence of *Flavivirus* infections, in part because of inconsistent surveillance and clinical misdiagnosis. Current laboratory methods are limited by lack of specificity and make conclusive diagnosis difficult (Roehrig et al., 2008). An accurate determination of baseline prevalence of *Flaviviruses* in the northern border regions of Kenya would contribute to appropriate risk evaluation or assessment which would guide preventive and control activities. Early detection of epidemics would be supported by improved clinical and laboratory diagnosis, coupled with knowing the geographic risk.

1.9 Conclusion

Arbovirus incidence and outbreaks in Africa are causing a strain on public health systems. Specifically, YF and dengue are two important viral hemorrhagic fevers which are re-emerging in many parts of Africa evidenced from recent outbreaks in Kenya, Tanzania, Somalia, South Sudan, Ethiopia, Central Africa Republic, Cameroon and Angola among others. These countries are largely dependent on foreign assistance to respond to these diseases and help with control and prevention. The recent increase in frequency of YF outbreaks in East Africa is unusual and of great concern to public health authorities in the region. This calls for a roll out of YF vaccination programs in the East African countries. However, there is little understanding of the

ecology of the virus that is vital for evaluating risks to facilitate cost-effective vaccination programs. Despite the availability of YF vaccines since the 1940s, large epidemics occurred in areas without a background of naturally acquired or artificial immunity. Dramatic upsurges in YFV activity occurred in Africa in the 1960s and the late 1980s each involving >100,000 cases. The first ever outbreak of YF in Kenya occurred in 1992/95 and was found to be from sylvatic cycle that spilled to rural human population. Recent outbreaks have also affected Uganda (2011, 2016, 2019 and 2020), and South Sudan and Ethiopia (2012–2013). Although the absence of an immune barrier in the human population is a key factor, the underlying reasons for virus amplification remain unclear, and are multifactorial, involving deterministic (vector density and competence, viral virulence), and stochastic factors.

While the factors leading to the re-emergence of these diseases are poorly understood, the need for more scientific research remains a priority to improve local capacities on arbovirus epidemiology and ecology. Currently, there is very limited information available on the mosquito species that inhabit the remote porous borders with countries reporting frequent outbreaks of YF like Uganda, Ethiopia, where populations travel across freely in West Pokot and Turkana counties in Northern Kenya. Nothing is known concerning the potential for YF and dengue transmission in this region, based on the potential vector mosquito species presence and their ability to be infected by, and transmit YF and DEN virus (competence). We set out to determine the abundance and diversity of *Aedes (Stegomyia)* species present in West Pokot County in Northern Kenya, including the evaluation of their blood feeding preferences. We determined the seroprevalence of the four most important flaviviruses, including; YFV, ZIKV, DENV and WNV, among asymptomatic human population, and determined the genetic variability and vector competence of *Ae. aegypti* for DENV serotype 2, all to assess the risk of transmission of

YF and DENV, and rule out other closely related *Flaviviruses*. These will guide informed decisions on YF immunization coverage, and improve our understanding of the ecology and role of *Aedes (Stegomyia)* mosquitoes in YFV and DENV transmission.

1.10 Hypothesis

1. The key *Stegomyia* species are neither abundant nor diverse, and are not anthropophilic; therefore, there is low human exposure to YF and DEN infections, and no variation in the genetics and competence of the key vectors in West Pokot and Turkana counties in Northern Kenya.

1.11 Objectives

1.11.1 Primary objective

To assess the entomologic and human exposure risk of yellow fever and dengue viruses by *Aedes stegomyia* species in Northern Kenya

1.11.2 Secondary objectives

1. To determine the presence, relative abundance and host blood feeding patterns of *Aedes stegomyia* species in Northern Kenya.
2. To determine the seroprevalence of YF and DEN among the human populations in West Pokot and Turkana counties
3. To determine the genetic variability and vector competence of *Aedes stegomyia* species from West Pokot and Turkana for DEN virus.

Chapter 2

THE OCCURRENCE, DIVERSITY AND BLOOD FEEDING PATTERNS OF POTENTIAL VECTORS OF DENGUE AND YELLOW FEVER IN KACHELIBA, WEST POKOT COUNTY, KENYA

2.1 Abstract

Yellow fever (YF) and dengue (DEN) viruses are important re-emerging mosquito-borne viruses sharing similar vectors and reservoirs. The last documented YF outbreak in Kenya occurred in 1992–95. However, YF virus is re-emerging in bordering countries including Uganda, Ethiopia and South Sudan with the potential for spread to the neighboring regions in Kenya. Dengue is endemic in Kenya with outbreaks being detected in various towns in the north and the coast. This study reports on the *Aedes (Stegomyia)* mosquito species occurrence, diversity, and blood feeding patterns, as means of measuring the risk of transmission of YF and DEN in Kacheliba sub-county, West Pokot County, which borders previous YF outbreak areas in eastern Uganda. Adult mosquitoes were collected using CO2-baited BG Sentinel traps at three time points during the rainy season. Mosquitoes were identified to the species level. Species abundance during the three sampling periods were compared, with emphasis on *Aedes aegypti* and other *Stegomyia* species, using generalized linear models that included mosquito diversity. Individually blood-fed mosquitoes were analyzed by DNA amplification of the 12S rRNA gene followed by sequencing to determine the source of blood meal. Overall, 8605 mosquitoes comprising 22 species in 5 genera were collected. Sampled *Stegomyia* species included *Ae. aegypti* (77.3%), *Ae. vittatus* (11.4%), *Ae. metallicus* (10.2%) and *Ae. unilineatus* (1.1%). *Ae. aegypti* dominated the blood-fed

specimens (77%, n=68) and were found to have fed mostly on rock hyraxes (79%), followed by goats (9%), humans and cattle (each 4%), with a minor proportion on hippopotamus and rock monitor lizards (each comprising 1%). Our findings reveal the presence of important *Stegomyia* species, which are known potential vectors of YF and DEN viruses. In addition, evidence of more host feeding on wild and domestic animals (hyrax and goat) than humans was observed. How the low feeding on humans translates to risk of transmission of these viruses, remains unclear, but calls for further research including vector competence studies of the mosquito populations for these viruses. This forms part of a comprehensive risk assessment package to guide decisions on implementation of affordable and sustainable vaccination (YF) and vector control plans in West Pokot County, Kenya.

2.2 Introduction

Yellow fever virus (YFV) and dengue virus (DENV) are among the most important mosquito-borne viruses of the family *Flaviviridae* and genus *Flavivirus*. Yellow fever virus was first isolated in West Africa in 1927 (Barrett and Higgs, 2007). The recent re-emergence of YFV with major outbreaks in countries bordering Kenya (Uganda, Ethiopia and South Sudan), and regionally in two countries - Angola (with cases of travelers being confirmed in Kenya) and the Democratic Republic of Congo - is of great concern to public health (Kraemer *et al.*, 2017). There is an estimated annual YF incidence of 200,000 cases, and 30,000 deaths, mostly in West and sub-Saharan Africa in which 33 countries and over 500 million people are at risk (WHO, 2014, Garske *et al.*, 2014, Kraemer *et al.*, 2017). In East Africa, the frequency of YF outbreaks is increasing after the first report in Kenya in 1992-95 (Sanders *et al.*, 1998, Reiter *et al.*, 1998), Sudan in 2003, 2005 and 2010 (Onyango *et al.*, 2004, WHO, 2005) and Uganda in 2011 and

2016 (WHO, 2011, WHO, 2016 and InterHealth Worldwide, 2016). The number of cases has increased over the past two decades (WHO, 2016, Kraemer *et al.*, 2017), perhaps because of decreased population immunity to this infection, deforestation, urbanization, population migration from rural to urban areas and vice versa, and the appearance of new vectors (Neiderud, 2015). It is therefore, crucial to carry out vector ecological studies to assess the risk of disease transmission in order to facilitate prioritization of rational regional vaccination programs considering the costs and availability of vaccines.

Dengue outbreaks have also increased in frequency in the region with reports in Tanzania, Somalia, Djibouti, Eritrea, Sudan and Kenya (Sang, 2007; Konongoi *et al.*, 2016; Vairo *et al.*, 2016). Dengue, one of the most important re-emerging arboviruses worldwide, was first reported in Kenya in 1982 during an outbreak in the coastal region (Johnson *et al.*, 1982). In 2011, a second dengue outbreak occurred in Mandera in North Eastern Kenya. Subsequently between 2013 and 2014, DEN outbreak occurred in Mombasa (Coastal Kenya), involving co-circulation of serotypes DEN-1, 2 and 3 (Konongoi *et al.*, 2016). With the expanding distribution of the principal vector, *Aedes aegypti*, combined with rapid population growth, unplanned urbanization, and increased international travel, extensive transmission of DENV is likely (Gubler, 2004).

Both YF and DEN share a niche in the ecosystem and focusing research efforts on both viruses is scientifically and financially prudent. DENV and YFV are primarily transmitted by *Stegomyia* mosquitoes. Both viruses originated in sylvatic cycles, are maintained in non-human primates and forest-dwelling *Aedes* mosquitoes, and have a history of successful emergence and sustained transmission among humans by *Ae. aegypti* (Hanley *et al.*, 2013). Emergence of urban YF could be mediated by the adaptation of sylvatic YFV to domestic vectors as has been previously

suggested for dengue, which shares similar vectors and vertebrate hosts. The adaptation of sylvatic vectors to rural or urban settings could similarly lead to emergence of these viruses in rural and urban areas (Weaver and Reisen, 2010, Coffey *et al.*, 2013). The scenario underscores the importance of understanding the types of vectors present in these ecological interfaces.

Mosquito biting frequency and how bites are distributed among humans and other vertebrate host species can influence transmission risk of a vector-borne disease (Adams and Kapan, 2009). An improved understanding of mosquito host blood feeding preferences would refine knowledge of the entomological processes supporting pathogen transmission and could reveal targets for minimizing risk and breaking pathogen transmission cycles (Harrington *et al.*, 2014). Many mosquito species express inherent traits in host preference, presence and abundance of available hosts. In addition, wild and domestic animal grazing areas create ideal conditions that facilitate virus amplification and transmission by mosquitoes from reservoir animals native to such habitats to susceptible domestic animals and human populations (O'Brien *et al.*, 2011). Thus, the need to determine the mosquito hosts blood feeding preferences in the area.

Surveillance for YF and dengue vectors is a critical component of assessing risk of transmission and outbreak occurrence. This requires knowledge on the distribution of potential vectors involved, including the critical aspects of their ecology such as abundance and their competence in transmitting and sustaining these viruses. Knowledge about their host blood feeding preferences can reveal information about potential reservoirs of disease and risk of transmission to susceptible populations. With more outbreaks being reported regionally, there is need to identify and evaluate vectors in locations in Kenya that border areas with reports of recent

outbreaks, which may be at increased risk of epidemic transmissions. This study reports findings on the vector species occurrence, composition and blood feeding patterns of the *Stegomyia* species, potential vectors, to assess the risk of transmission of YF and DEN in West Pokot County which borders recent YF outbreak areas in Uganda.

2.3 Methods

2.3.1 Ethical Considerations

Approval for this study was provided by Kenya Medical Research Institute (KEMRI) Centre Scientific Committee (CSC), the KEMRI Scientific and Ethics Review Unit (SERU), (under protocol number KEMRI-SERU 2787) and Faculty of Health Sciences Research Ethics, University of Pretoria (Protocol number 491/2017).

2.3.2 Study site

This study was conducted in West Pokot County, Kacheliba sub-county, in Kenya. The county borders Uganda (Figure 3), which experienced the most recent outbreaks of YF in 2011 and 2016 in areas bordering the county. The area is characterized by hot and dry weather most of the year with an annual mean temperature of about 10°C to 30 °C. Rainfall is erratic and unpredictable with the annual mean ranging from about 300-400 mm; falling to less than 150 mm in the arid central parts. The driest months are January through to March, while the wettest are April through June, with the other months receiving little or no rainfall. Bush density also decreases with decrease in rainfall (Climate –data.org, 2018). The mean daily rainfall during the sampling

period was 3.94mm, 1.3mm and 5.55mm in the months of May 2015, December 2015 and May 2016, respectively.

The human activity in West Pokot, whose population is approximately 512,690 (KNBS, 2009), is mainly nomadic pastoralism. The herders usually move between Kacheliba and the neighboring Uganda in search of water, food and pasture. This practice usually puts them at risk of exposure to YF and other exotic viruses, and has potential to lead to cross boarder exchange of diseases between the two countries. Currently, however, there is increasing tendency for the people to migrate to urban centers, where they adopt sedentary lifestyles creating a suitable environment for breeding of mosquitoes. There is also risk of viremic persons returning, to initiate local transmission in Kenya with potential for more widespread activity.

Mosquitoes were collected from peridomestic areas, in small villages in the remote areas of Kacheliba sub-county. These included Pkotong, Shaba, Kacheliba and Kokibei hills that comprise of caves which form good habitats for rock dwelling animals such as rock hyraxes. In addition, there are several rock pools that collect water creating ideal conditions for mosquito breeding long after the rains. Pkotong, Shaba and Kacheliba hills are between 1 and 2 km apart and all neighbour the Kacheliba town. Kokibei is situated about 15 kms northwest of Kacheliba town and is situated right on the Kenya-Uganda border. Sampling was also conducted in Tabadany, Nge'nge'chwa and Kanyerus all characterized by flat terrain, open grasslands with presence of small bushes, shrubs and acacia trees. While Tabadany and Nge'nge'chwa are proximal to Kacheliba town, Kanyerus is situated about 30 kms southwest of Kacheliba town, and like Kokibei, lies close to the Kenya-Uganda border.

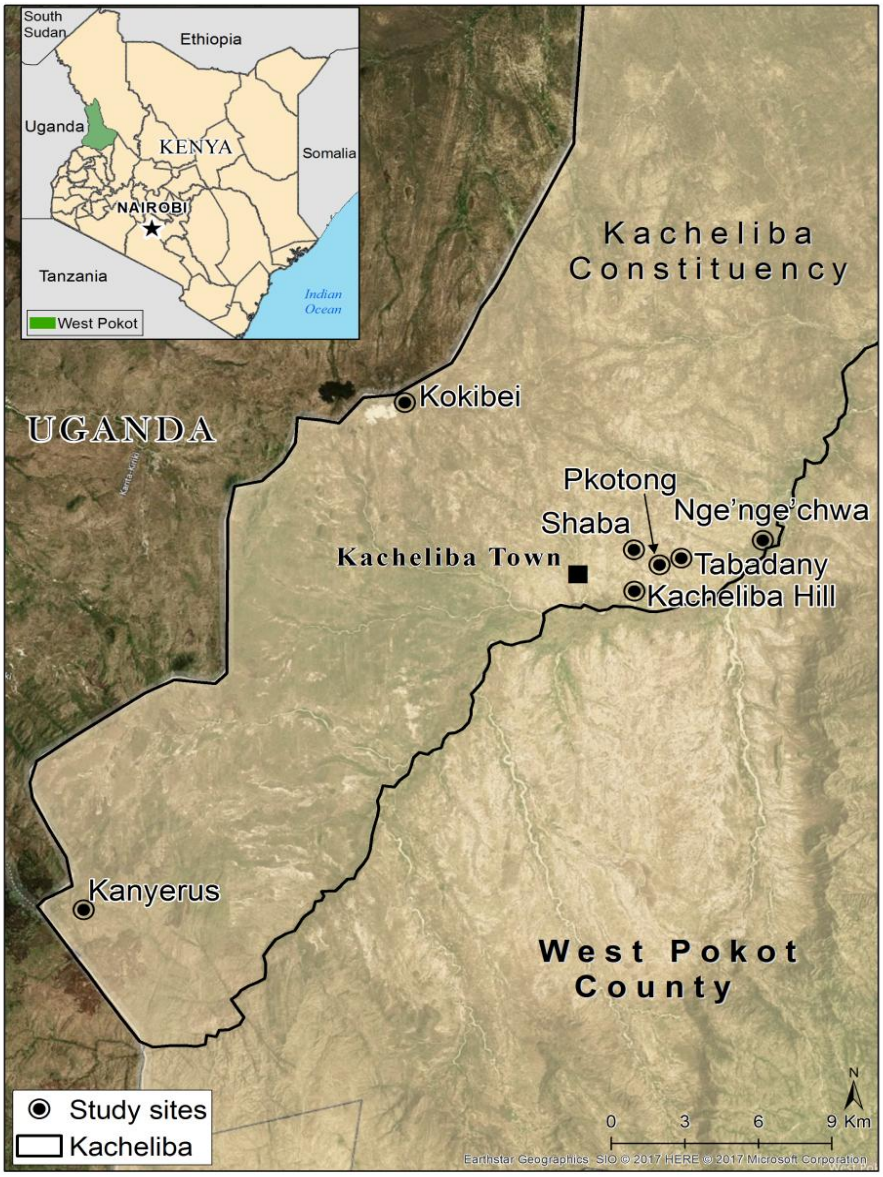

Figure 3: Map showing the sites sampled within Kacheliba sub-County during the May 2015, December 2015 and May 2016 sampling periods

2.3.3 Mosquito sampling, processing and identification

Mosquito sampling was conducted for 10 days during each of two long rainy seasons (May 3-12, 2015 and May 14-23, 2016) and the short rainy season of 2015 from December 10-19. Adult

collection was done using BG Sentinel (BioQuip Products, Rancho Dominiguez, CA, USA) traps (ten traps per site) baited with CO_2 supplied in the form of dry ice and dispensed in Thermos flasks (~2L capacity). The ten traps were set at 0600 hours and retrieved at 1800 hours each day during the sampling period (Figure 4). The collected mosquitoes were sorted into vials by collection site, date, and stored in liquid nitrogen awaiting transportation to the *icipe* Emerging Infectious Diseases (EID) laboratory. The collected mosquitoes were identified by morphology using taxonomic keys (Edwards, 1941; Huang, 2004). Blood fed mosquitoes were stored singly for mosquito host blood meal analysis.

Figure 4: Photograph showing mosquito sampling. **(A)** BG sentinel trap placed on the ground and hanging over is a 2 litre Igloo thermos flask containing dry ice as a source of CO_2. **(B)** Observation of the BG sentinel trap captures. (Source: Edith Chepkorir)

2.3.4 Host blood feeding preference

Individual blood fed mosquito abdomens were separated from the rest of the body using a scalpel which was sterilized before every successive decapitation. Each separated abdomen was transferred into a sterile 1.5 ml microcentrifuge tube and triturated in 500 µl of phosphate buffered saline (PBS). The DNA was then extracted using the DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer's instructions. The DNA obtained was used as the template in a standard polymerase chain reaction (PCR) to amplify the 12S mitochondrial rRNA gene using published primer sets 12S3F [5′-GGGATTAGATACCCCACTATGC3′] and 12S5R [5′-TGCTTACCATGTTACGACTT-3′], a target used for vertebrate host blood meal identification (Roca *et al.*, 2004). All amplification products (~ 500 bp) were resolved in 1% agarose gels stained with ethidium bromide. All amplicons were purified using a PCR purification kit (Promega) following the manufacturer's instructions. Bidirectional Sanger sequencing was done by Inqaba (Pretoria, South Africa). The sequences were cleaned and analysed using Molecular Evolutionary Genetics Analysis version 6.0 (MEGA). Sequences were assigned to particular species by Blastn analysis of the GenBank DNA sequence database (National Center for Biotechnology Information (NCBI), 2008) and the Barcode of Life Data (BOLD) Systems database (http://www.boldsystems.org/views/login.php). Positive identification and host species assignment were based on exact or near exact matches (>98%).

2.3.5 Statistical analysis

Mosquitoes were pooled for each trapping period: May 2015, December 2015 and May 2016. We determined mosquito diversity for each sampling period, by estimating the Shannon diversity

index ('diversity') using the vegan package (Oksanen *et al.*, 2015) in R version 3.3.1 (R development Core Team). We compared the abundance patterns (response variable) for all mosquito species and for individual species (*Ae. aegypti*, *Ae. metallicus* and *Ae. vittatus*) across the sampling periods (predictor variable) using generalized linear models (GLMs) with negative binomial error structure. We focused on *Ae. aegypti*, *Ae. metallicus* and *Ae. vittatus* as they were fairly represented across the sampling periods. The diversity trends across the sampling periods were compared after log-transformation. This was ascertained using normal GLMs with diversity specified as main factor. The short rain season (December 2015) was taken as a reference sampling period. Data normality for diversity data was confirmed by performing Shapiro–Wilk tests on model residuals. All tests were performed at 5% significance level.

2.4 Results

2.4.1 Presence and abundance of *Aedes stegomyia* species

A total of 8605 mosquitoes, comprising 22 species in 5 genera, were collected during the different sampling periods (Table 1). *Aedes aegypti* was most predominant and was represented in all sampling periods. There were significantly more *Stegomyia* mosquito captures during the May 2016 sampling period; 12-fold higher compared to May 2015 and 3-fold higher compared to December 2015 (Table 2.1). Overall, the region was dominated by five genera *Aedes*, *Anopheles*, *Culex*, *Mansonia* and *Coquillettidia*. The *Aedes* genus was most represented in the mosquito captures (11 species), followed by *Anopheles* genus (5 species), and then *Culex* (4 species). *Mansonia* and *Coquillettidia* were each represented by 1 species (Table 1).

Table 1: Summary of mosquito species in Kacheliba sub-county sampled during the May 2015, December 2015 and May 2016 sampling periods

	Sampling Periods		
	Long rain season	**Short rain season**	**Long rain season**
Species	May 2015	December 2015	May 2016
Aedes aegypti	385	1551	4726
Aedes vittatus	4	115	323
Aedes metallicus	3	6	295
Aedes hirsutus	1	0	13
Aedes mcintoshi	1	0	3
Aedes sudanensis	0	0	7
Aedes tarsalis	0	0	23
Aedes tricholabis	0	0	2
Aedes unilineatus	0	0	21
Aedes chaussieri	0	1	0
Aedes species	0	0	2
Anopheles coustani	0	0	1
Anopheles funestus s.l.	0	7	369
Anopheles gambiae s.l.	1	2	16
Anopheles nili	0	18	0
Anopheles rufipes	0	0	5
Coquillettidia aurites	0	0	1
Culex pipiens	14	14	375
Culex tigripes	0	0	1
Culex univittatus	4	1	26
Culex zombaensis	0	0	263
Mansonia uniformis	0	0	5
Total	**413**	**1715**	**6477**

Data for Pkotong, Shaba and Kacheliba hill, and that of Tabadany and Nge'nge'chwa sites, were merged because of their proximity to each other, the sites were presented as K1 and K2, respectively. A higher number of potential YFV and DENV vectors were collected in K1 (Pkotong, Shaba and Kacheliba hill sites), compared to K2 (Tabadany and Nge'nge'chwa) and K3 (Kokibei) (Table 2).

Table 2: Distribution of potential DENV and YFV vectors collected from sampling sites during different sampling periods

Sites	Season	*Ae. chaussieri*	*Ae. aegypti*	*Ae. metallicus*	*Ae. unilineatus*	*Ae. vittatus*
K1 (Pkotong, Shaba and Kacheliba hill)	Long rain, May 2015	0	384	3	0	4
	Short rain, Dec 2015	1	1551	6	0	115
	Long rain, May 2016	0	3521	218	20	284
	Total	**1**	**5456**	**227**	**20**	**403**
K2 (Tabadany and Nge'nge'chwa)	Long rain, May 2015	0	1	0	0	0
	Short rain, Dec 2015	0	0	0	0	0
	Long rain, May 2016	0	1186	69	0	39
	Total	**0**	**1187**	**69**	**0**	**39**
K3 (Kokibei)	Long rain, May 2015	0	0	0	0	0
	Short rain, Dec 2015	ns	ns	ns	ns	ns
	Long rain, May 2016	0	19	8	0	0
	Total	**0**	**19**	**8**	**0**	**0**

ns- not sampled

Mosquito diversity based on Shannon diversity index showed significant differences across the sampling periods ($p < 0.0001$). Mean diversity ranged from 0.38 to 1.12, with the highest being 3-fold higher compared to the lowest. The highest species diversity was recorded during the May 2016 sampling period (Figure 5).

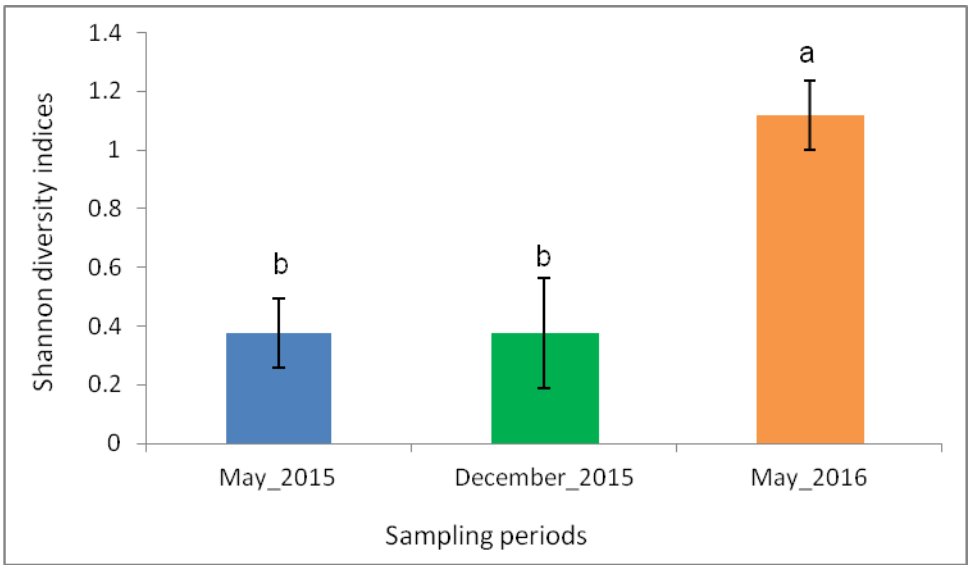

Figure 5: Shannon diversity indices across the three sampling periods. There was significantly high species diversity during the May 2016 sampling period. Error bars denote the standard error. Bars followed by different letters (a and b) denote significant difference at 5% significance level.

Total mosquito abundance and the abundance of potential vectors of DEN and YF, showed significant variation across the sampling periods (Table 3).

Table 3: Negative binomial results comparing the abundance of the mosquitoes across the sampling periods

	Total Abundance			*Aedes aegypti*			*Aedes metallicus*			*Aedes vittatus*		
	estimate ± se	Z value	*p*-value	estimate ± se	Z value	*p*-value	estimate ± se	Z value	*p*-value	estimate± se	Z value	*p*-value
December 2015- short rain	1			1			1			1		
May 2015- Long rain	-1.760 ± 0.584	-3.016	0.003 **	-1.730± 0.708	-2.442	0.015 *	-1.030± 0.793	-1.299	0.194	-3.695± 1.275	-2.899	0.004 **
May 2016- Long rain	0.741 ± 0.554	1.337	0.181	0.526 ± 0.673	0.782	0.4343	3.307± 0.536	6.176	<0.001***	0.445± 1.119	0.398	0.691

se - Standard error

*- Significant codes; *, p<0.05; **, p<0.01; ***, p<0.001

Highest abundance and diversity were recorded during the May 2016 collection, while the lowest abundance was recorded in May 2015 (Figure 6). The lowest diversity was recorded in the short rain season sampling, December 2015.

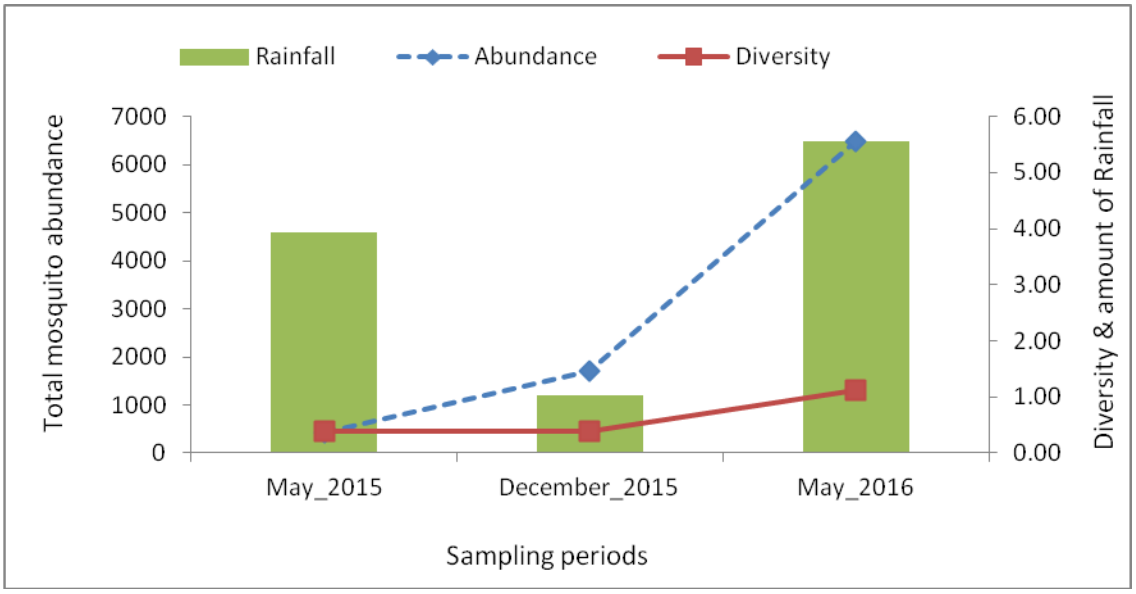

Figure 6: The total abundance and mean species diversity of mosquitoes collected, and the amount of rainfall (mm) during the sampling periods. The abundance and diversity increased with increased amount of rainfall

Among host-seeking females of potential DENV and YFV vectors, *Ae. aegypti* was predominant (n=6662, 77.42%), followed by *Ae. vittatus* (n=442, 5.14%) and *Ae. metallicus* (n=304, 3.53%) (Figure 7). *Aedes unilineatus* had the least number sampled only during the long rain season in May 2016.

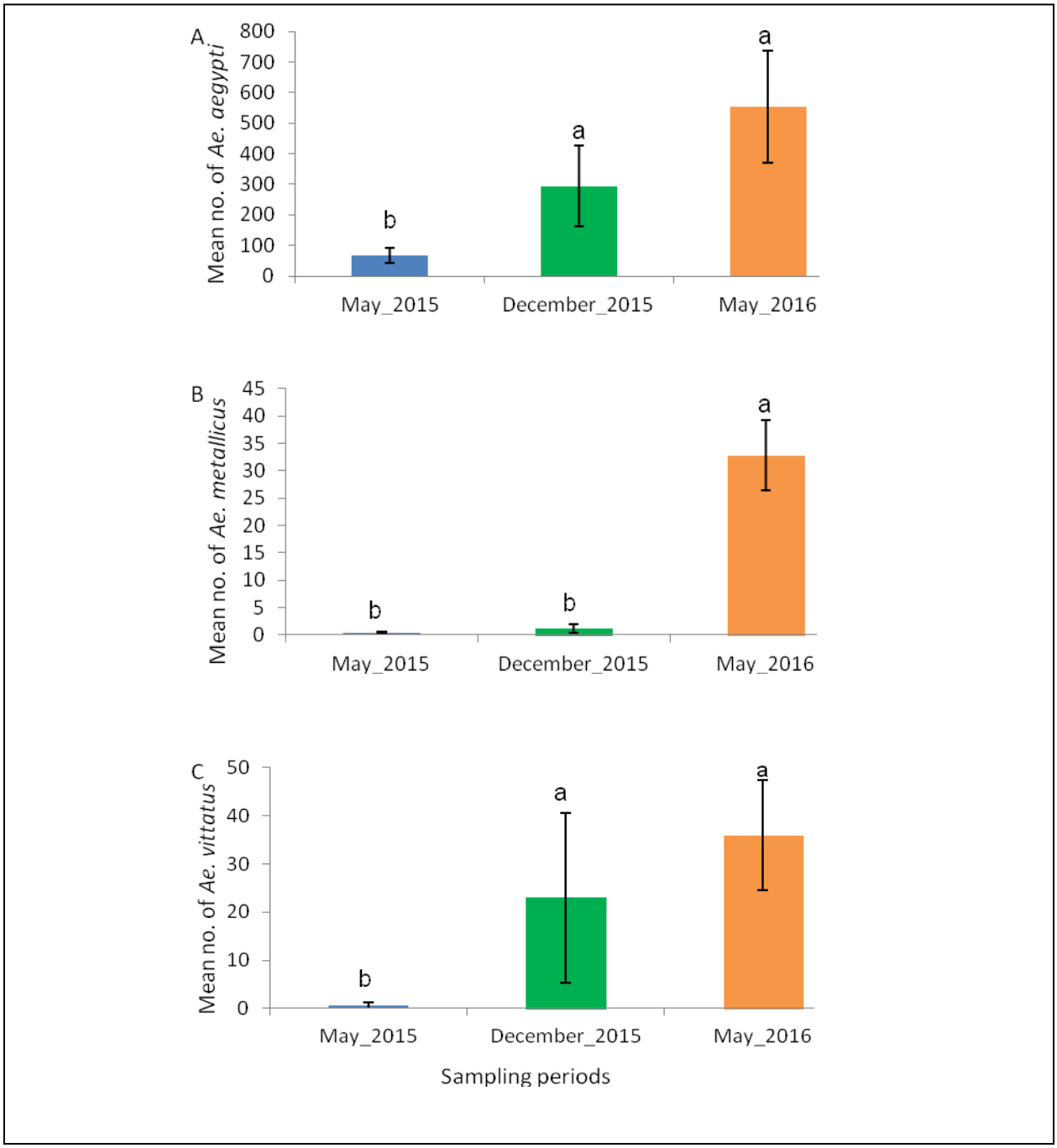

Figure 7: The abundance of (A) *Ae. aegypti*, (B) *Ae. metallicus* and (C) *Ae. vittatus*, potential vectors of DENV and YFV across different sampling periods in West Pokot, Kenya. Error bars

denote the standard error. Bars followed by different letters denote significant difference at 5% significance level.

2.4.2 Host blood feeding preference

A total of 104 blood-fed mosquitoes were processed and only eighty eight (88) were successfully identified. The potential vectors of DENV and YFV, found were: *Ae. aegypti*: 68, *Ae. metallicus:* 9, *Ae. unilineatus*: 1 and *Ae. vittatus*: 10 (Figure 8). The majority of *Ae. aegypti* had fed on rock hyrax (n=54, 79%), followed by goats (n=6, 9%), humans and cattle (n=3, 4% each), and two had fed on hippopotamus and rock monitor lizard (each n=1, 1%). Most *Ae. vittatus* collected had fed on goats (n=4, 40%), followed by cattle (n=3, 30%), human (n=2, 20%) and sheep (n=1, 10%). As for *Ae. metallicus*, a similar number had fed on goat and sheep (n=3, 33.3%), while 22.2% (n=2) had fed on rock hyrax and 11.1% (n=1) on human. The single blood-fed *Ae. unilineatus* had fed on rock hyrax.

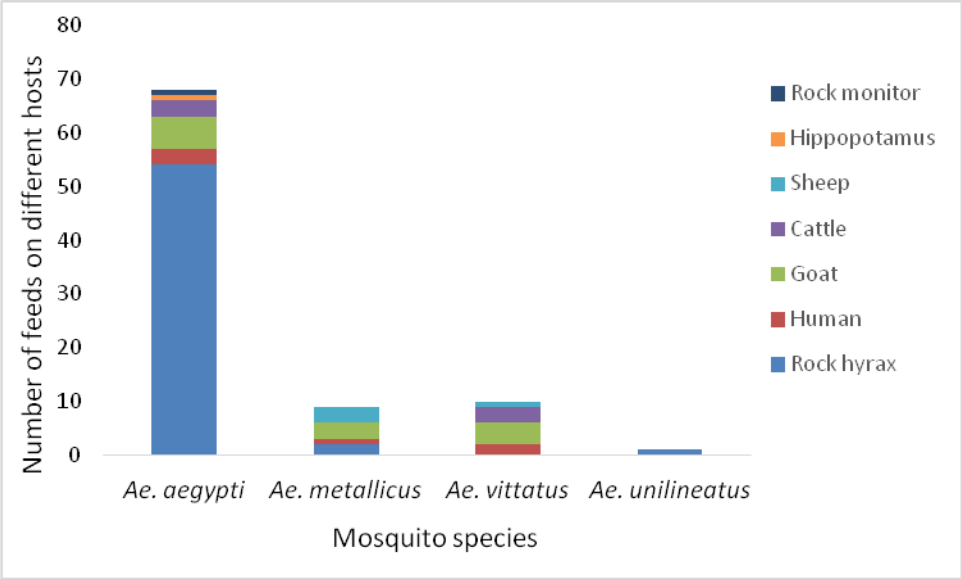

Figure 8: Host blood feeding patterns for the potential vectors of DENV and YFV in West Pokot County, Kenya. Total number analyzed, n=88

2.5 Discussion

The surveillance of vectors (vector presence and their associated bionomic parameters, including abundance and blood feeding preferences) is essential for assessing the risk of pathogen transmission and occurrence of outbreaks. Our findings show that the diurnal mosquito fauna in the study area in West Pokot County, Kenya, bordering Uganda, is dominated by *Stegomyia* species, notably *Ae. aegypti*, *Ae. vittatus* and *Ae. metallicus*. These species are of medical significance having been implicated in the transmission of YFV/DENV in different settings (Cordellier, 1991; Barrett and Higgs, 2007; Ngoagouni *et al.*, 2012; Hanley *et al.*, 2013). Therefore, their presence in the study area would signify potential risk of transmission of YF and DEN viruses.

Total mosquito abundance correlated with a period of highest rainfall (May 2016). The effect of rainfall on mosquito abundance is well recognized (Arum *et al.*, 2015; Agha *et al.*, 2017a; Agha *et al.*, 2017b). Rainfall increases the availability of natural and artificial sites for mosquito breeding and development. Risk of virus transmission and potential for outbreaks has been found to be high during such periods of abundant rainfall (Aitken *et al.*, 1968; de Kruijf, 1972). An analogous pattern was observed for individual species including *Ae. aegypti*, *Ae. metallicus* and *Ae. vittatus*. Coincidentally, mosquito diversity and species richness showed a significant variation across the sampling periods and correlated with the period of highest rainfall.

The mosquito fauna of the study area seems to be less diverse compared to other sites in Kenya (Arum *et al.*, 2015; Agha *et al.*, 2017a; Agha *et al.*, 2017b). Diversity estimates may however be affected by the type of sampling tool deployed. BG Sentinel traps, set during the day, were used in the current study, these traps are known to have bias for *Stegomyia* species (Barrera *et al.*, 2013). To overcome this limitation, traps were baited with CO_2, which is a universal attractant for most hematophagous insects, including mosquitoes (Dekker *et al.*, 2005). Human landing collection may be the most effective method for sampling sylvatic *Aedes* (Diallo *et al.*, 2012), but it was not possible to obtain ethical clearance for this collection method for the current study. Although our approach was aimed at collecting *Aedes* species of potential importance to DENV/YFV epidemiology, a combination of other tools including larval sampling may be more appropriate to determine species diversity. Overall, our study provides a useful baseline and first report on the diurnal mosquito fauna inhabiting the ecology of our study area in Kenya.

Host feeding pattern is an important component of vectorial capacity. This can reveal information about vector-host contact rates and potential reservoirs important in the amplification of a virus, and disease epidemiology. Based on our findings, the mosquitoes displayed a more zoophagic tendency, with most *Stegomyia* feeding on rock hyraxes, goats, cattle and sheep, and few feeding on humans. This may be due to the availability of particular hosts, such as rock hyraxes, which are abundant compared to the other hosts especially around the breeding areas. However, other possible adaptive preferences cannot be discounted. We showed that *Ae. aegypti, Ae. metallicus* and *Ae. vittatus* feed on multiple hosts, including humans, indicating a potential to transmit zoonotic viruses to humans. This assertion will require

data on their ability to transmit the viruses, and vector competence studies that are ongoing. Additional sampling and testing of adult samples for viruses using a combination of methods including culture and sequencing would be valuable.

Aedes aegypti is the main DEN/YF virus vector in most urban settings (Barrett and Higgs, 2007; Ngoagouni *et al.,* 2012; Hanley *et al.,* 2013; Agha *et al.,* 2017a). The zoophilic tendency observed for this species in this study, although striking, mirrors previous findings reported in West Africa (Diallo *et al.,* 2012) associated with the sylvatic form, *Ae. aegypti* subspecies *formosus*. The likelihood that this form dominates the sylvatic setting characteristic of our study sites remains high, although further studies are recommended to ascertain this. The low feeds on humans may suggest low risk of transmission of these viruses especially YFV by this species. In the Kenyan YF outbreak of 1992–1993, *Ae. aegypti* was not the incriminated vector; rather, *Ae. africanus, Ae. bromeliae* and *Ae. keniensis* were identified as vectors through virus isolation during the outbreak (Reiter *et al.,* 1998). Furthermore, in the sylvatic setting in West Africa, this species is considered not to play any role in the transmission of important viruses such as Chikungunya virus (Diallo *et al.,* 2012). This form is thought to feed mainly on wild animals, especially non-human primates (Diallo *et al.,* 2012), important in the natural transmission cycle of YFV and DENV. Surprisingly, we did not find any blood feeds on non-human primates in any of the mosquito species examined, although they were observed to be present in sampling area. Seasonal migratory patterns among non-human primates in search of food are well recognized (Alberts and Altmann, 2001). The lack of blood feeding from this host could be attributed to mosquito trapping coinciding with periods when the non-human primates had migrated to other

localities within the vast West Pokot County or across the border to Uganda, probably in search of food.

Chapter 3

SEROLOGICAL EVIDENCE OF *FLAVIVIRUS* CIRCULATION IN HUMAN POPULATIONS IN NORTHERN KENYA: AN ASSESSMENT OF DISEASE RISK 2016-2017

3.1 Abstract

Yellow fever, dengue, West Nile and Zika viruses are re-emerging mosquito-borne *Flaviviruses* of public health concern. However, the extent of human exposure to these viruses and associated disease burden in Kenya and Africa at large remains unknown. We assessed the seroprevalence of yellow fever and other *Flaviviruses* in human populations in West Pokot and Turkana Counties of Kenya. These areas border Uganda, South Sudan and Ethiopia where recent outbreaks of yellow fever and dengue have been reported, with possibility of spillover to Kenya. Human serum samples collected through a cross-sectional survey in West Pokot and Turkana Counties were screened for neutralizing antibodies to yellow fever, dengue-2, West Nile and Zika virus using the Plaque Reduction Neutralization Test (PRNT). Seroprevalence was compared by county, site and important human demographic characteristics. Adjusted odds ratios (aOR) were estimated using Firth logistic regression model. Of 877 samples tested, 127

neutralized with at least one of the four flaviviruses (14.5, 95% CI 12.3–17.0%), with a higher proportion in Turkana (21.1%, n = 87/413) than in West Pokot (8.6%, n = 40/464). Zika virus seroprevalence was significantly higher in West Pokot (7.11%) than in Turkana County (0.24%; $\chi 2$ $P < 0.0001$). A significantly higher yellow fever virus seroprevalence was also observed in Turkana (10.7%) compared to West Pokot (1.29%; $\chi 2$ $P < 0.0001$). A high prevalence of West Nile virus was detected in Turkana County only (10.2%) while dengue was only detected in one sample, from West Pokot. The odds of infection with West Nile virus was significantly higher in males than in females (aOR = 2.55, 95% CI 1.22–5.34). Similarly, the risk of Zika virus infection in West Pokot was twice higher in males than females (aOR = 2.01, 95% CI 0.91–4.41). Evidence of neutralizing antibodies to West Nile and Zika viruses indicates that they have been circulating undetected in human populations in these areas. While the observed yellow fever prevalence in Turkana and West Pokot Counties may imply virus activity, we speculate that this could also be as a result of vaccination following the yellow fever outbreak in the Omo river valley, South Sudan and Uganda across the border.

3.2 Introduction

Yellow fever virus (YFV), dengue virus (DENV), West Nile virus (WNV) and Zika virus (ZIKV) are important mosquito-borne *Flaviviruses* that have a potential to cause severe disease and mortalities in humans with economic and ecological consequence (Huang et al., 2014; Nyamwaya et al., 2016). Approximately 831 million people in Africa are at risk of infection with at least one of these viruses (Weetman et al., 2018). Epidemiologic studies on these mosquito-borne viruses remain a priority given their risk of global spread and high epidemic potential.

There have been no cases of yellow fever (YF) in Kenya since the first and last ever documented outbreak in 1992-95 (Sanders et al., 1998; Reiter et al., 1998). However, recent re-emergence of the virus with major outbreaks in border countries of Uganda, Ethiopia and South Sudan (Onyango et al., 2004; WHO, 2011; WHO, 2016; Lilay et al., 2017) and regionally in Angola and Democratic Republic of Congo has become a major public health concern (Kraemer et al., 2017). Dengue is currently endemic in parts of Kenya, with outbreaks reported in some specific geographic zones in the coast and northern frontier of Kenya associated with dengue Serotypes 1-3, and neighboring countries like South Sudan, Somalia and Tanzania (Ellis et al., 2015; Konongoi et al., 2016). West Nile on the other hand, was first isolated in Uganda in 1937 and is now one of the re-emerging zoonotic mosquito-borne pathogens whose occurrence and geographical range continues to spread (Nyamwaya et al., 2016).

Zika virus was first discovered in Uganda in 1947 (Dick et al., 1952), and since then, there has been limited data available on its circulation in the region. Previously, virological and immunological evidence suggested that although ZIKV was distributed widely in Africa and Asia, Zika fever was not a disease of substantial concern to human beings because only 14 cases had been documented worldwide (Filipe et al., 1973; Simpson, 1964). In 2016 extensive outbreaks of the virus were reported in the Americas and the association with an increased incidence of microcephaly and Guillain-Barre cases (Kindhauser et al., 2016) that led to the World Health Organization (WHO) declaration of Zika virus as a public health emergency of international concern. The increased detection of Zika virus worldwide and its association with increasingly large outbreaks of disease has heightened awareness of this emerging mosquito-transmitted pathogen (Wikan and Smith, 2016).

There is limited epidemiologic knowledge about the presence and spread of arboviral diseases in northern Kenya. Surveillance capacities are lacking, with most resources for study and control of these viruses being focused on epidemic periods. As such, the magnitude of human exposure and the burden of these important *Flavivirus* diseases in this region remain poorly understood. West Pokot and Turkana Counties are located in areas that border countries that have had and reported outbreaks of these *Flaviviruses* in recent times and therefore the risk of virus spread from neighboring countries remains high. This study sought to determine the seroprevalence of yellow fever, dengue, West Nile and Zika viruses among the human populations in the border locations of West Pokot and Turkana Counties.

3.3 Methods

3.3.1 Ethical approval

This study was approved by Kenya Medical Research Institute (KEMRI) Scientific and Ethics Review Unit (SERU) (under protocol number KEMRI-SERU 2787) and University of Pretoria (Protocol number 491/2017). All adult participants provided written informed consent and assent for child/minor participants. Only those who consented or assented were included in the study.

3.3.2 Study sites and study population

This was a cross sectional population based study conducted in Turkana and West Pokot Counties of Kenya. Six sites each were selected and sampled in West Pokot County: Kacheliba sub-county (Kokipei, Sangakai, Kanyerus, Shaba, Longarkau and Pkotong) and Turkana County,

Lokitoung sub-county (Elelea, Prigan, Kare-Edome, Nayanaesanyati, Old Gunner and Lowarengak) (Figure 9). Turkana County borders three high risk countries for YF, based on past and recent outbreaks. These include Ethiopia to the North, along the Omo River Valley, which experienced one of the largest YF outbreaks in East Africa in 1968 (Serie et al., 1968a) and recently in 2013 (Lilay et al., 2017). Also, to the North is South Sudan and Uganda to the west. West Pokot borders Uganda which experienced an outbreak of YF in 2011 and 2016 (WHO, 2011; WHO, 2016). All the three neighboring countries have experienced YF outbreaks in locations that are close to Turkana and Kacheliba (West Pokot; Figure 9).

The human populations in West Pokot are mainly nomadic pastoralists although there is increasing tendency for them to move to market centers and become sedentary. In Turkana, they are mainly nomadic pastoralists and tend to look for pasture in areas surrounding the lake, and some engage in fishing. As of 2009, the population of Turkana was 855,399 and West Pokot 512,690 (KNBS, 2009). The livelihoods of these communities are characterized by lack of most basic amenities like water, health care, education and even food. Corollary, they sometimes migrate as far as the neighboring countries of Uganda, South Sudan and Ethiopia in search of water and pasture, a practice that may put them at risk of exposure to YF and other exotic viruses.

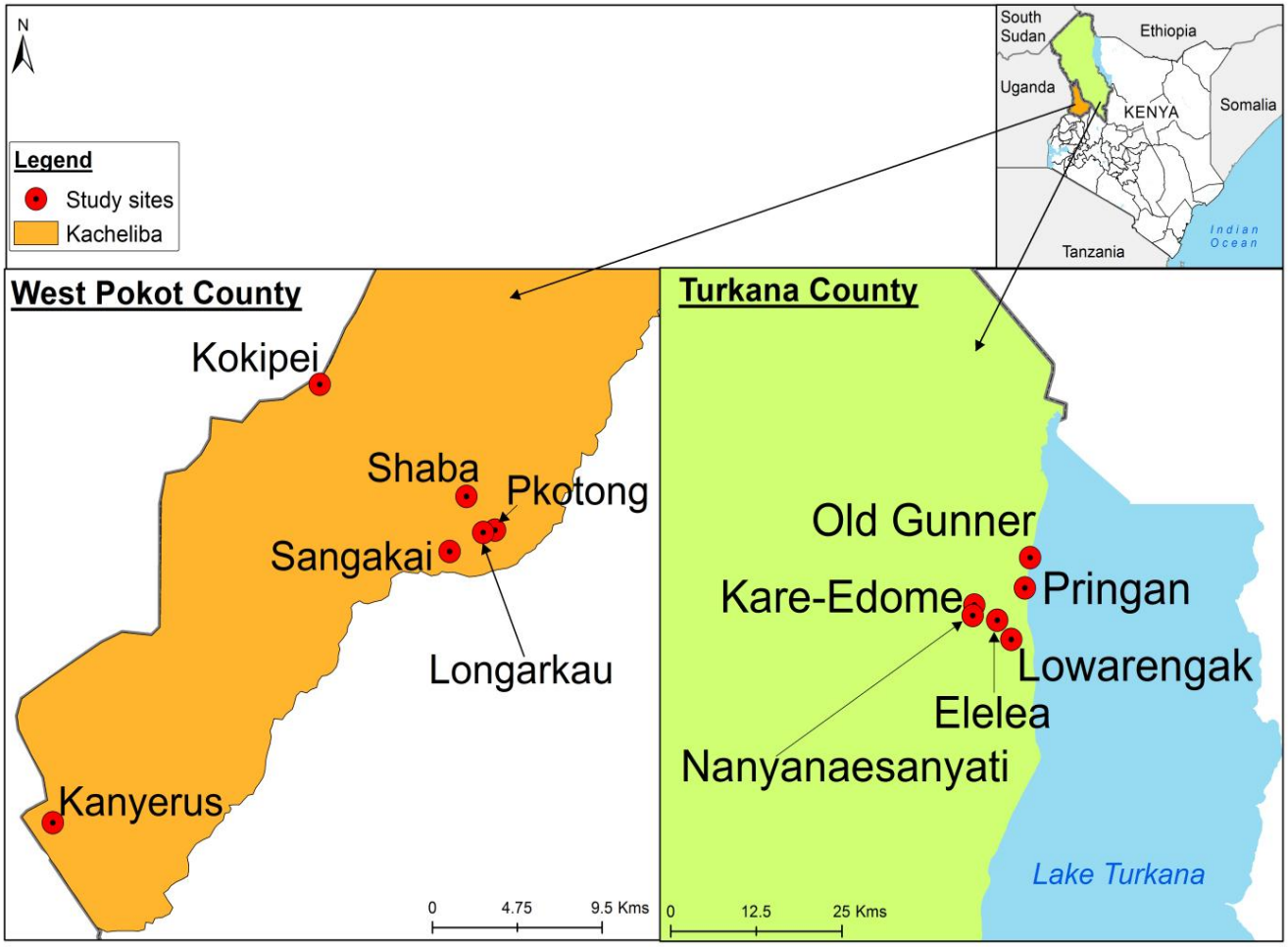

Figure 9: Map of Kenya showing the study sites in West Pokot and Turkana Counties

3.3.3 Sample collection

Human blood samples were collected through a cross-sectional study designed into village clusters based on administrative boundaries, in West Pokot and Turkana Counties, Kenya (Figure 9) in February 2016 and August 2017, respectively. Blood samples were collected from a randomized asymptomatic human population within the village clusters. Both males and females aged between 12 and 95, who consented/assented to participate in the study, were included. An individual was excluded if he/she declined to give assent/consent. Gender, age, place of

residence, occupation, YF vaccination and travel history were recorded for each study participant. A total volume of 5ml of blood was collected from each study participant using vacutainer tubes with a clot activator. The tubes with blood samples were left to stand at room temperature for 10 minutes and then placed on sample carrier at 2-8°C for transport to the field laboratory. At the field laboratory, the serum was processed by centrifugation at a relative centrifugal force (RCF) of 112 for 3 minutes, then aliquoted into 1ml volumes, placed in two sterile cryovials. All the serum samples collected were preserved in liquid nitrogen and transported to the Martin Luscher Emerging infectious Disease (MLEID) laboratory at the International Centre of Insect Physiology and Ecology (*icipe*) Duduville Campus in Nairobi, where they were stored at -80°C until tested.

3.3.4 Plaque Reduction Neutralization Assay (PRNT)

Samples were retrieved from the freezer, aliquoted in volumes of 30µl, heat inactivated at 56°C for 30 minutes and then tested for neutralizing antibodies to YF (YFXSMB), dengue-2 (008/01/2012), West Nile (AMH005348) and Zika (MR766) viruses in two-fold serum dilutions from 1:10 to 1:320 using PRNT90 (Table 4). Respective virus isolates were diluted to a standard concentration that gave 20-50 plaques. Appropriate 10-fold dilutions of serum samples were prepared in maintenance media (Minimum Essential Media Eagles, Sigma with Earle's salts and reduced $NaHCO_3$) supplemented with 2% heat-inactivated fetal bovine serum (FBS), 2% L-glutamine, 2% penicillin/amphotericin B (Sigma-Aldrich, St. Louis, MO), starting with a seropositivity threshold of 1:10. The serum dilutions were added to microcentrifuge tubes containing the standard concentration of the diluted virus and incubated for 1hr at 37°C. The virus–antibody mixture was inoculated on a 24-well plate containing confluent Vero cell

monolayer (VERO E6) and incubated for 1hr for virus adsorption. After adsorption process, an overlay of methylcellulose (2.5%) (Sigma) mixed with 2X Minimum Essential Medium (Sigma), was added into the wells. Between 6 – 14 days depending on the virus tested, the plaques were fixed using 10% formalin (Sigma), stained with 0.25% crystal violet (Sigma) diluted in absolute ethanol and the plaques counted manually as described previously (Tigoi et al., 2015; Odhiambo et al., 2015). Endpoint titres were determined as the reciprocal of the highest serum dilution giving ≥ 90% reduction in plaque counts (Roehrig et al., 2008) (Figure 10).

Table 4: Details of the viruses used for PRNT

Virus strain	Year of Isolation	Country	Host	Passage history (P)
Yellow fever (YFXSMB)	1992	Kenya	Human	p3
Dengue-2 (008/01/2012)	2012	Kenya	Human	p3
West Nile (AMH005348)	2010	Kenya	Mosquito	P3
Zika (MR766)	1947	Uganda	Rhesus Monkey	P2*

*Sourced commercially.

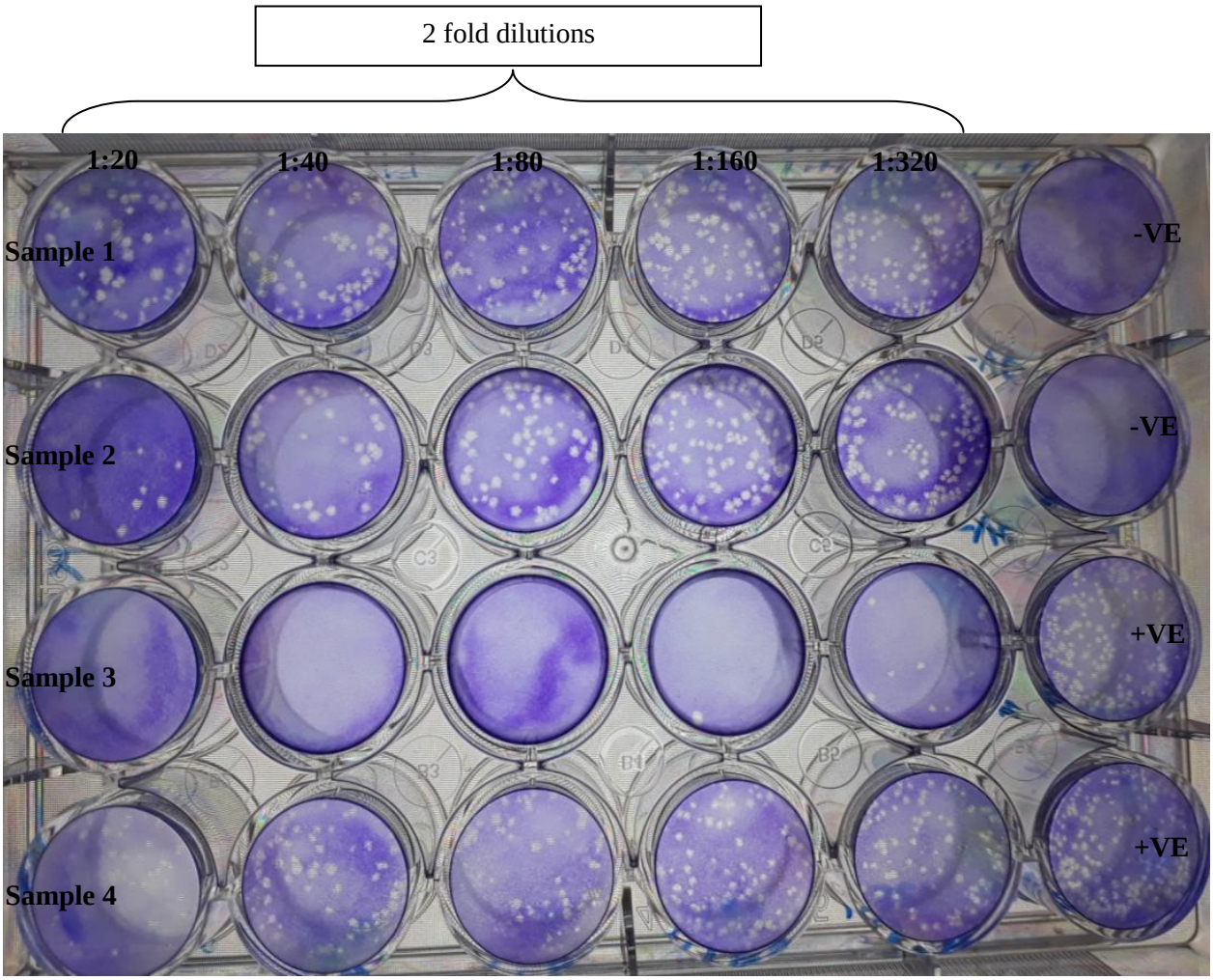

Figure 10: Picture showing comparative Plaque Reduction Neutralizing Test performed on a 24-well plate

3.3.5 Statistical analysis

Field data captured were stored in a password-protected database linked to the laboratory results, and were analyzed using R version 3.3.1 (R Development Core Team, 2008). First, we characterized the study participants with respect to exposure to the *Flaviviruses* tested. Reported prevalence was compared by county, site and important demographic characteristics such as sex,

age, occupation and YF vaccination status. Comparisons of proportions and evaluation of heterogeneity of the seropositive proportions across the independent variables listed above were performed using Chi Square test. The 95% confidence intervals (CIs) for the proportions positive for a virus were estimated using the Agresti-Coull method (Brown et al., 2001). Next, we fitted for each of the three most prevalent *Flaviviruses* with a frequency of six or more positive samples (i.e., YFV in both West Pokot and Turkana; ZIKV in West Pokot; WNV in Turkana), a multiple logistic regression model with covariates as site, sex, age in years, occupation, whether the participant was a herdsman, and whether the participant reported to have or have not received YF vaccination. As the data were sparse, Firth's logistic regression (Firth, 1993) was chosen. Firth's approach reduces the bias in maximum likelihood (ML) estimates of coefficients when binary events are rare by penalizing the likelihood function using Jeffreys invariant prior. In addition, it also allows for the computation of reliable, finite estimates of coefficients in the case of separation, where ML estimation fails (Heinze & Schemper, 2002). All tests were performed at 5% significance level.

3.4 Results

3.4.1 Demographic profile of study participants

Table 5 shows the distribution of the study participants with respect to their demographic characteristics for the individual and combined counties. A total of 877 serum samples were collected; 464 from West Pokot and 413 from Turkana. Of these samples, 64% (565/877) were from females and 36% (312/877) males. In both counties, there were more female participants than males. The males were often away from the homesteads as their occupational activities (fishing and farming) kept them away at the time of enrolment and sampling. The sampled

participants' age ranged from 13 to 92 years, and their mean (median) age was 39 years (35 years). About three in five study participants were farmers, but this varied across the counties as almost all the participants (98.5%) in West Pokot were farmers. About 23% of the study participants were housewives or female domestic servants, 9% fishermen, 2.4% were either teachers or students, and the rest were involved in other economic activities. Most participants (42%) indicated they had not been vaccinated against YF, 36% did not know their YF vaccination status, and 22% indicated that they had been vaccinated.

Table 5: Demographic characteristics of study participants from Kacheliba in West Pokot and Lokitoung in Turkana Counties

Characteristic	West Pokot County		Turkana County		Combined	
	N	%	N	%	N	%
All	464	100.0	413	100.0	877	100.0
Sex						
Female	324	69.8	241	58.4	565	64.4
Male	140	30.2	172	41.6	312	35.6
Age (years)						
13-19	14	3	48	11.6	62	7.1
20-29	101	21.8	139	33.7	240	27.4
30-39	106	22.8	86	20.8	192	21.9
40-49	77	16.6	38	9.2	115	13.1
50+	166	35.8	102	24.7	268	30.6
Occupation						
Farmer	457	98.5	71	17.2	528	60.2
Wife/housegirl	0	0.0	198	47.9	198	22.6
Fisherman/vendor	0	0.0	82	19.9	82	9.4
Teacher/student	4	0.9	17	4.1	21	2.4
Other	3	0.7	45	10.9	48	5.5
Herdsman						
No	425	91.6	373	90.3	798	91.0
Yes	39	8.4	40	9.7	79	9.0
Yellow Fever vaccinated						
No	192	41.4	176	42.6	368	42.0
Yes	182	39.2	11	2.7	193	22.0
don't know	90	19.4	226	54.7	316	36.0

3.4.2 Prevalence of antibodies against *Flaviviruses* in West Pokot and Turkana counties

Of 877 samples tested, 127 (14.5%, 95%CI 12.3-17.0%) were positive for antibodies against at least one of the four *Flaviviruses*. This proportion was, however, higher in Turkana (21.1%) than in West Pokot (8.6%; P<0.0001). Figure 11 presents plaque reduction neutralization test results stratified by county. First, the figure shows that in general, the virus to which the most neutralizing antibodies were present was YFV, followed by WNV, ZIKV and DENV. Second,

there was variability in the serologic prevalence against the *Flaviviruses* between the two counties. Whereas DENV neutralizing antibodies were detected in one sample (0.22% prevalence) from West Pokot only, WNV antibodies were detected in Turkana only (42/413, prevalence=10.2%). While YFV and ZIKV antibodies were detected in samples from both counties, YFV was significantly more prevalent in Turkana (10.7%) than West Pokot (1.29%; χ^2 P<0.0001). The converse was true for ZIKV.

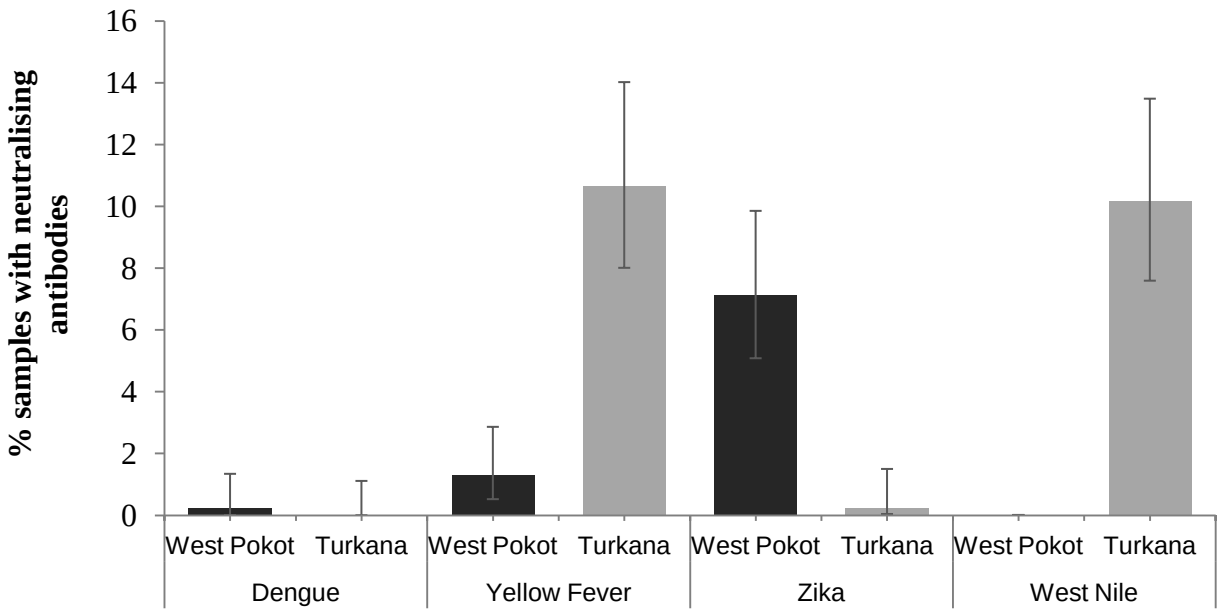

Figure 11: Seroprevalence of antibodies against the various Flaviviruses presented separately for each county. The error bars indicate Agresti-Coull 95% confidence intervals. The number of samples tested was 413 in Turkana and 464 in West Pokot

Table 6 summarizes PRNT results across six sites within each county. There was variability in *Flavivirus* antibodies prevalence across the sites within as well as between the counties. Yellow fever antibodies prevalence ranged from 0% in Kokipei, Shaba, Longarkau, and Pkotong to

about 4% in Kanyerus in West Pokot. In Turkana, YF antibodies prevalence varied from about 7% in Kare Edome to about 20% in Elelea. For West Nile virus antibodies, which were detected in Turkana only, the prevalence varied from 8% in Prigan to 11.1% in Elelea. Also samples from only two sites had neutralizing antibodies to three viruses detected. These were Sangakai in West Pokot (DEN, YF, and ZIK) and Elelea in Turkana (YF, ZIK, and WN).

Table 6: Flavivirus PRNT90 endpoint titre results for sites in Kacheliba, West Pokot County and Lokitoung, Turkana County

Site	Number of samples tested	Number of samples (%) positive of each Flavivirus			
		Dengue	Yellow Fever	Zika	West Nile
West Pokot					
Kokipei	100	0 (0.00)	0 (0.00)	8 (8.00)	0 (0.00)
Sangakai	66	1 (1.52)	2 (3.03)	6 (9.09)	0 (0.00)
Kanyerus	113	0 (0.00)	4 (3.54)	13 (11.50)	0 (0.00)
Shaba	73	0 (0.00)	0 (0.00)	3 (4.11)	0 (0.00)
Longarkau	60	0 (0.00)	0 (0.00)	1 (1.67)	0 (0.00)
Pkotong	52	0 (0.00)	0 (0.00)	2 (3.85)	0 (0.00)
Total	464	1 (0.22)	6 (1.29)	33 (7.11)	0 (0.00)
Turkana					
Elelea	63	0 (0.00)	12 (19.05)	1 (1.59)	7 (11.11)
Prigan	25	0 (0.00)	2 (8.00)	0 (0.00)	2 (8.00)
Kare Edome	61	0 (0.00)	4 (6.56)	0 (0.00)	6 (9.84)
Nayanaesanyati	69	0 (0.00)	6 (8.70)	0 (0.00)	7 (10.14)
Old gunner	56	0 (0.00)	5 (8.93)	0 (0.00)	5 (8.93)
Lowarengak	139	0 (0.00)	15 (10.79)	0 (0.00)	15 (10.79)
Total	413	0 (0.00)	44 (10.65)	1 (0.24)	42 (10.17)

Prevalence of the three most prevalent *Flaviviruses* (ZIKV, YFV and WNV) varied by age group in both counties (Figure 12). In West Pokot, the prevalence of antibodies against ZIKV was highest among those in the age group 13-19 years and lowest among the age group 20-29 years, though the difference was not significant. Yellow fever neutralizing antibodies were more among the age group 30-39 years and less among those aged 50+ years, though the difference was not significant. In Turkana County, the prevalence of WNV was highest among those aged ≥50 years (χ^2 P =0.048) and lowest among the age group 20-29 years. Zika virus antibodies were only detected in the 50+ years age group in Turkana County. In general, variation between seroprevalence against *Flaviviruses* was quite limited between age groups.

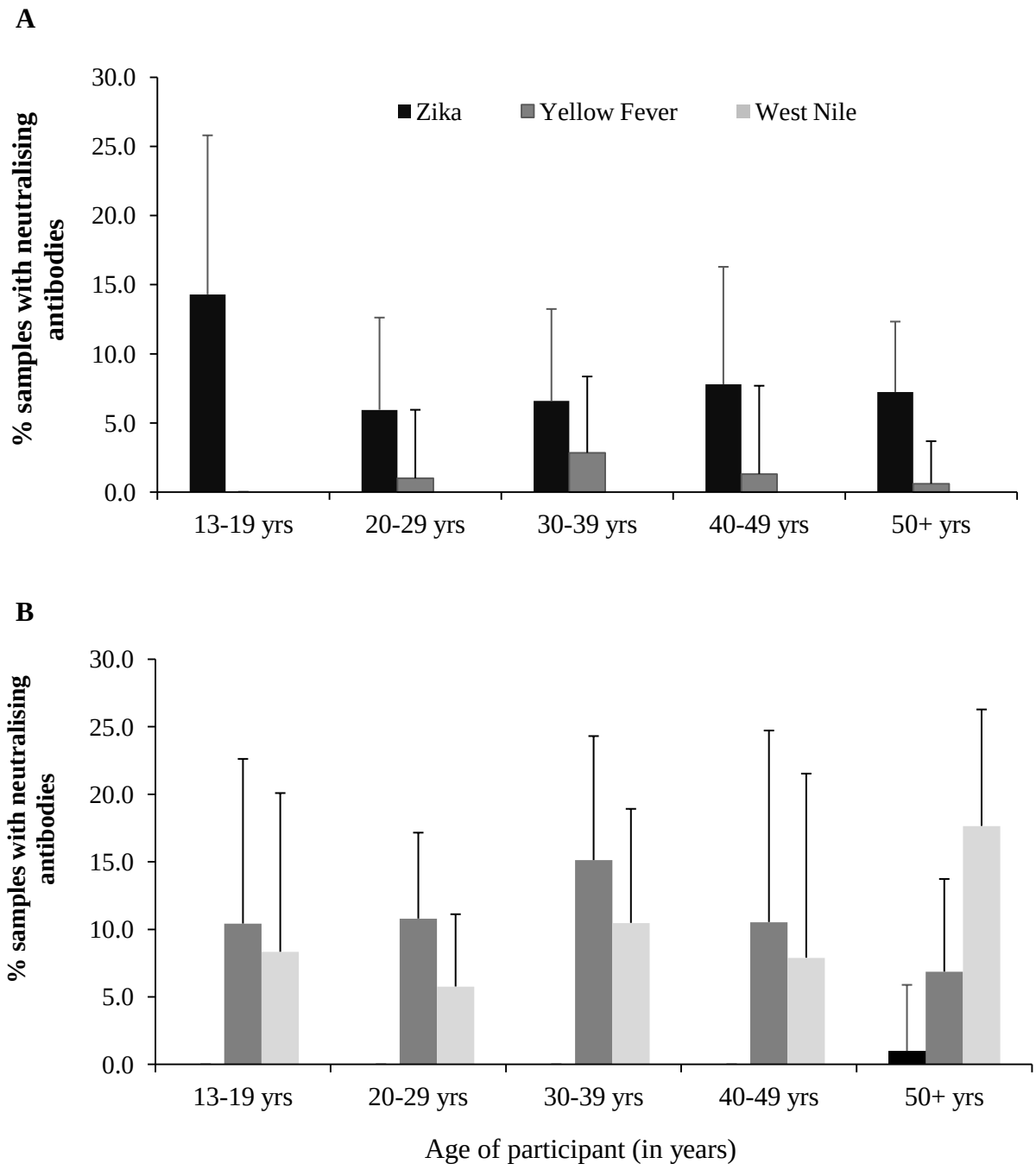

Figure 12: Proportions positive for three most prevalent viruses (ZIK, YF and WN) by age group for (A) West Pokot (n=464) and (B) Turkana (n=413) counties. The error bars indicate Agresti-Coull 95% confidence intervals

Flaviviruses that had more than 30 samples neutralizing with either of the three most prevalent viruses for each county (i.e., West Pokot: ZIKV (n=33); Turkana: YFV (n=44), WNV (n=42) by demographic characteristics are presented in Table 7. The result shows that significantly higher numbers of males than females were exposed to West Nile and Zika virus in Turkana and West Pokot counties, respectively. West Nile virus exposure was significantly represented in the age group 50+ years compared to the others in Turkana County.

Table 7: Prevalence of Zika virus (West Pokot County) and Yellow Fever, West Nile (Turkana County) by demographic characteristics

Characteristic	West Pokot			Turkana					
	Zika			Yellow fever			West Nile		
	Number positive	Percent Positive	χ^2 Pvalue	Number positive	Percent Positive	χ^2 Pvalue	Number positive	Percent Positive	χ^2 Pvalue
All	33	7.1		44	10.7		42	10.2	
Sex									
Female	18	5.6		29	12.0		14	5.8	
Male	15	10.7	0.047	15	8.7	0.282	28	16.3	0.001
Age (years)									
13-19	2	14.3		5	10.4		4	8.3	
20-29	6	5.9		15	10.8		8	5.8	
30-39	7	6.6		13	15.1		9	10.5	
40-49	6	7.8		4	10.5		3	7.9	
50+	12	7.2	0.844	7	6.9	0.502	18	17.6	0.048
Occupation									
Farmer	33	7.2		8	11.3		12	16.9	
Wife/housegirl	0	0.0		22	11.1		13	6.6	
Fisherman/vendor	0	0.0		6	7.3		9	11.0	
Teacher/student	0	0.0		4	23.5		1	5.9	
Other	0	0.0	0.762	4	8.9	0.388	7	15.6	0.086
Herdsman									
No	28	6.6		41	11.0		34	9.1	
Yes	5	12.8	0.147	3	7.5	0.496	8	20.0	0.030
Yellow Fever vaccinated									
No	12	6.3		18	10.2		19	10.8	
Yes	10	5.5		1	9.1		2	18.2	
don't know	11	12.2	0.106	25	11.1	0.951	21	9.3	0.595

3.4.3 Firth's logistic regression model results

Table 8 shows the adjusted odds ratios (aORs) and the associated 95% confidence intervals estimated using Firth's logistic regression model. The data show that in Turkana County, males were about three times more likely to be infected with WNV than females (aOR = 2.55, 95%CI 1.22-5.34). There was a two-fold likelihood of WNV infection among the age group 50+ years compared to the age group 13-19 years. There was no difference in YF seroprevalence in Turkana by site, sex, age group and vaccination status. In West Pokot County, YF infection was eight times likely among study participants from Sangakai (aOR=8.24, 95%CI 0.31-220.15) and Kanyerus (aOR = 8.32, 95%CI 0.40-172.04), and two times highly likely in participants from Longarkau (aOR=2.02, 95%CI 0.04-110.05) and Pkotong (aOR=2.02, 95%CI 0.04-110.5). The odds of infection with ZIKV in West Pokot was twice as high in males compared to females (aOR = 2.01, 95%CI 0.91-4.41).

Table 8: Comparison of Flavivirus prevalence by site, demographic parameters and history of YF vaccination from Firth's multiple logistic regression model

Variable	West Pokot (N=464)				Turkana (N=413)				
	Yellow fever (n=6)		Zika (n=33)			Yellow Fever (n=44)		West Nile (n=42)	
	aOR (95%CI)	Pvalue	aOR (95%CI)	Pvalue	Variable	aOR (95%CI)	Pvalue	aOR (95%CI)	Pvalue
Site					Site				
Kokipei	Reference		Reference		Elelea	Reference		Reference	
Sangakai	8.24 (0.31-220.15)	0.208	0.77 (0.23-2.60)	0.669	Prigan	0.42 (0.10-1.79)	0.240	0.98 (0.21-4.69)	0.982
Kanyerus	8.32 (0.40-172.04)	0.170	1.10 (0.41-2.93)	0.853	Kare Edome	0.32 (0.10-1.04)	0.058	0.94 (0.28-3.17)	0.925
Shaba	1.46 (0.03-77.85)	0.851	0.45 (0.12-1.70)	0.241	Nayanaesanyati	0.36 (0.12-1.07)	0.066	1.21 (0.37-3.92)	0.749
Longarkau	2.02 (0.04-110.5)	0.731	0.24 (0.04-1.46)	0.122	Old gunner	0.39 (0.12-1.22)	0.106	0.88 (0.24-3.14)	0.838
Pkotong	2.02 (0.04-104.34)	0.728	0.36 (0.08-1.67)	0.191	Lowarengak	0.39 (0.15-0.99)	0.047	1.45 (0.49-4.31)	0.505
Sex					Sex				
Female	Reference		Reference		female	Reference		Reference	
Male	0.40 (0.04-4.62)	0.466	2.01 (0.91-4.41)	0.084	male	0.84 (0.41-1.72)	0.631	2.55 (1.22-5.34)	0.013
Age group					Age group				
13-19	Reference		Reference		13-19	Reference		Reference	
20-29	0.82 (0.03-24.26)	0.910	0.35 (0.07-1.81)	0.209	20-29	1.26 (0.41-3.84)	0.686	0.76 (0.22-2.66)	0.663
30-39	1.76 (0.08-38.73)	0.721	0.42 (0.09-2.07)	0.288	30-39	1.59 (0.52-4.84)	0.414	1.43 (0.41-4.92)	0.574
40-49	1.06 (0.04-30.49)	0.975	0.45 (0.09-2.31)	0.337	40-49	1.03 (0.26-4.01)	0.970	1.09 (0.24-4.94)	0.912
50+	0.39 (0.01-12.26)	0.595	0.34 (0.07-1.59)	0.169	50+	0.72 (0.22-2.38)	0.589	2.10 (0.66-6.66)	0.206
Herdsman					Herdsman				
No	Reference		Reference		No	Reference		Reference	
Yes	3.16 (0.2-48.99)	0.411	1.16 (0.36-3.72)	0.806	Yes	0.93 (0.27-3.28)	0.915	1.40 (0.52-3.74)	0.508
Yellow fever vaccinated					Yellow fever vaccinated				
No	Reference		Reference		No	Reference		Reference	
Yes	0.61 (0.09-3.91)	0.599	1.05 (0.42-2.60)	0.924	Yes	0.81 (0.13-5.13)	0.819	1.70 (0.34-8.57)	0.519
Don't know	1.45 (0.22-9.79)	0.701	2.90 (1.11-7.60)	0.030	Don't know	1.21 (0.61-2.41)	0.586	0.75 (0.37-1.53)	0.427

3.5 Discussion

The present study reports on the serological evidence of human exposure to *Flaviviruses* in West Pokot and Turkana counties of northern Kenya. Overall, we found that 14.5% (n=127, 95%CI 12.3-17.0%) of the samples were positive for at least one flavivirus (ZIKV, YFV, DENV-2, WNV). However, there were variations in the rates of human exposure for the individual viruses between the counties and across sites. Such variation in prevalence by site or even region is not uncommon in Kenya (Ochieng et al., 2015; Vu et al., 2017; Grossi-Soyster et al., 2017). At the county level, seroprevalence ranged from 0.22% for DENV in West Pokot to 10.7% for YFV in Turkana. Much higher exposure of specific flavivirus has been observed, of up to 60% for DENV in coastal Kenya (Ochieng et al., 2015; Sutherland et al., 2011). Such variability in specific flavivirus as observed in our study and elsewhere in Kenya suggests exposure is likely conditioned by a myriad of ecological and human factors such as vector species and abundance, climate and weather patterns, environmental features, occupational activities, degree of mobility among others.

West Nile virus was detected in Turkana (42/413, 10.2%), but not in West Pokot. This difference could relate partly to ecological variation between the two areas. West Nile virus is associated with *Culex* mosquitoes, the virus having been previously isolated in some *Culex* species in Kenya (Miller et al., 2000; LaBeaud et al., 2011; Ochieng et al., 2013). It is possible the mosquito fauna especially the *Culex* spp. abundance and diversity varies between the areas. Grossi-Soyster (Grossi-Soyster et al., 2017) found high risk of both alphaviruses and flaviviruses primarily associated with households near the Lake area in Western Kenya. In this case, the

presence of a lake (Lake Turkana) may encourage high diversity of bird species and breeding of *Culex* species, which are important determinants of the epidemiology of WNV (Nyamwaya et al., 2016). This may possibly account for higher exposure rates in humans in Turkana than West Pokot. Proximity to Lake Turkana may also influence the economic activities of the people living in the area, such as fishing or grazing livestock. Older men spend more time away from home fishing, taking care of the livestock and looking for pasture in areas around the lake. Such outdoor activities could predispose them to more mosquito infectious bites. This could explain the obvious disparity in exposure rates among males compared to females, even though more samples were analyzed from the latter relative to the former. In fact, males were about three times more likely to be infected with WNV than females (aOR = 2.55, 95%CI 1.22-5.34). Additionally, adult males aged 50+ had more exposure, consistent with previous findings (Sutherland et al., 2011). This alludes to higher risk due to advanced age; however, exposure in the 13-19 age group is indicative of ongoing transmission of this virus. Evidence of isolation of the virus from *Culex* spp. in this ecology (Ochieng et al., 2013) lends support for active virus transmission.

Neutralizing antibodies against YFV were detected in samples from both counties, although significantly higher in Turkana than West Pokot ($P<0.0001$). Site specific variation in exposure rates was also evident ranging from 7% in Kare Edome to about 20% in Elelea in Turkana County. It has been about three decades since the last documented outbreak of YF in Kenya in the neighboring Rift Valley (Sanders et al., 1998; Reiter et al., 1998). The exposure in the 13-19 age group, although higher in 30-39 age category, could indicate low-level ongoing transmission and persistent exposure in this region. The participants' responses to their YF vaccination status

made it difficult to determine the definite source of infection for YF reactive samples. Record of vaccination status was based on self-reporting which could not be verified, as no vaccination cards were provided during survey. However, the presence of YF neutralizing antibodies in these two counties may be attributable in part to the vaccinations that were rolled out in 2003 in response to the outbreak that was reported in the neighboring South Sudan (Onyango et al., 2004). Additionally, anecdotal information from the Kenyan Ministry of Health and Sanitation suggests that, some of the population received YF vaccination in Uganda in response to the 2011 YF outbreak (WHO, 2011), while they had crossed the borders to Uganda for pasture and trade. However, the level of immunity seems too low for a population that was vaccinated so recently to forestall an outbreak from a neighboring country. If vaccination was carried out to prevent an outbreak, then a seroprevalence of 10% is a far cry from the desired protective herd immunity level (80%) and should be enhanced. We also cannot rule out the circulation of YF in low levels in these regions, because of cross border travel by this population to outbreak areas in the neighboring countries in search of pasture and trade contributing to this high seroprevalence. Our findings somehow point to active circulation of YFV in low levels in these regions; similar exposure rates have been observed in other regions of Kenya (Sutherland et al., 2011).

Only one sample in West Pokot was reactive to DENV and none from Turkana. This in part may suggest low human exposure to this serotype in this region. Previous studies have highlighted the prevalence of DEN-2 serotype in Kenya through serological studies (Mease et al., 2011; Konongoi et al., 2016; Vu et al., 2017). Nonetheless, human exposure to the other serotypes (1, 3-4) has been reported (Sutherland et al., 2011). While we employed PRNT which is the gold standard for determining serological specific among viruses, cross reactivity of human sera,

however, occurs with all four DENV serotypes (Nawa et al., 2000) and even with other flaviviruses. The limitation of our analysis to only a single serotype (DEN-2), suggests more studies are needed to determine the exact burden of DEN in this region and possibly establish the serotype distribution.

The results in the current study suggest circulation of Zika in both counties, yet there have never been reported or confirmed cases of ZIKV in Kenya. The prevalence of Zika was significantly higher in West Pokot than in Turkana County. Coincidentally, the highest seroprevalence was recorded in Kanyerus (Figure 8), proximal to the Uganda border. The virus was first detected in Uganda at Zika forest in 1947 (Dick et al., 1952). Exposure could be influenced by the degree of human mobility between this site and across border into Uganda. As nomadic pastoralists, long distance movement of livestock in search of pasture or water is common practice especially during drought. Few studies have reported data on human exposure to ZIKV in Kenya, the last dating >40 years (Geser et al., 1970). The high prevalence among the age group 13-19 years possibly indicates evidence of active circulation of this virus in this ecology, which may have gone unnoticed due to lack of detection facilities. This calls for focused arboviral surveillance to forestall severe consequences of the virus emergence and incriminate the important vectors to enhance preparedness in dealing with potential outbreaks.

Our sampling approach focused only on asymptomatic population and could not tell whether the exposure represented here did manifest clinically. It is well known that clinical presentations of arboviral diseases are often highly non-specific, commonly indistinguishable from each other and from other common tropical diseases like malaria (Sang and Dunster, 2001). The lack of

diagnostic capability for arboviruses being routinely implemented in any health facility in the study area makes tracking of clinical cases very difficult, outside the confines of research studies. The need for continuous monitoring remains a priority given the unanticipated emergence or reemergence of arboviral diseases in recent years mainly in the form of outbreaks. Active surveillance including virus activity in vectors would help to shed light on the specific vectors involved and risk trajectory. Such studies could employ emerging technologies such as Next Generation Sequencing (NGS) to identify and characterize even unknown pathogens that could potentially be of human health impact.

This study has some limitations that should be considered. Our sampling approach targeted asymptomatic population in a cross-sectional study design. There was no clinical information related to these infections in the study population. Further studies should include clinical disease monitoring and should be extended to include young children and pregnant women, as in the case of ZIKV which has been found to be associated with congenital birth defects, including microcephaly in the developing fetus (Mlakar et al., 2016). In PRNT analysis, we employed only DEN-2 serotype which affects definite conclusions that can be drawn regarding DEN exposure and other serotypes in the study areas. Also, while we used the Firth's logistic regression model to reduce bias in estimates and to overcome the computational problems experienced with the conventional logistic regression model, the low frequencies of the viruses – as has been reported in the literature – does make it difficult to associate the prevalence with the risk factors.

Chapter 4

GENETIC VARIABILITY OF *AEDES AEGYPTI* POPULATIONS FROM WEST POKOT AND TURKANA COUNTIES IN NORTHERN KENYA, AND THEIR ABILITY TO TRANSMIT DENGUE VIRUS

4.1 Abstract

East Africa and Kenya in particular, have witnessed unprecedented emergence and re-emergence of diseases caused by arboviral pathogens in epidemic proportion in the recent past. Less is known about the presence of the two forms *Aedes aegypti* and whether their differences in viral competence exist especially for dengue virus in West Pokot and Turkana counties. The main objective of this study was to compare the genetic diversity and distribution of the forms of *Ae. aegypti* from both areas and assess the vector competence of the species for dengue-2 virus. Mosquitoes were collected from peridomestic areas at the selected sites using adult and larval collection tools. Mitochondrial sequence data was employed to determine the genetic variability of *Ae. aegypti* collected from West Pokot and Turkana counties. The barcode region of the cytochrome oxidase subunit 1 (*COI*) gene was amplified using published *COI* primers. Vector competence experiments were performed to determine the susceptibility of *Ae. aegypti* from this region, for DENV-2. The neighbor joining phylogeny based on *COI* gene sequences revealed the presence of two lineages. In one, all samples from Turkana and a proportion from West Pokot formed a lineage, clustered with the sylvatic *Ae. aegypti formosus*. The second mosquito lineage exclusively caught from West Pokot County, clustered with the domestic form of *Ae. aegypti*. Interestingly, *Ae. aegypti* population from West Pokot were more susceptible to dengue-2 virus

compared to the mosquito population from Turkana County, though there was no disseminated infection. The study confirms the presence of both subspecies of *Ae. aegypti* in the Northern region and the difference in their susceptibility to dengue-2 virus varied significantly.

4.2 Introduction

Aedes aegypti (Linnaeus) is a major vector of many emerging and re-emerging arboviral pathogens of public health significance including yellow fever, chikungunya, zika and dengue viruses (Gubler, 2002; Yergolkar et al., 2006; Charrel et al 2007; Li et al., 2012). East Africa, and Kenya in particular, have witnessed unprecedented emergence and re-emergence of diseases caused by these pathogens in epidemic proportion in the recent past (Sanders et al., 1998; Reiter et al., 1998; Konongoi et al., 2016; Mboera et al., 2016; Konongoi et al., 2018). Surveillance of the vector is critical for effective prevention and control of these arbovirus infections in humans (Ellis & Barrett, 2008; Gubler, 2004). Entomologic parameters are routinely monitored as measures of transmission risk of these pathogens including adult vector abundance, infection rates and blood meal host feeding patterns (Agha et al., 2019).

Immature infestation patterns are important attributes for *Aedes*-borne transmission risk relying on estimation of traditional Stegomyia indices (House Index-HI, Container Index-CI and Breteau Index-BI) (Lutomiah et al., 2016; Mboera et al., 2016; Agha et al., 2017a). However, these indices or abundance patterns may not always translate to risk of transmission of these pathogens (Pham Thi et al., 2017) as the competence of a vector to a given virus may be linked to specific populations (Chepkorir et al., 2014; Agha et al., 2017c). Thus, genotype-based surveys of vectors have been advocated in arbovirus surveillance studies (Vazeille et al., 2016; Pham Thi et al., 2017).

Aedes aegypti is often regarded as a homogeneous species in its role as a disease vector. However, this species has been found to be rich in genetic, morphological and ecological variation (Tabachnick et al., 1979; Powell & Tabachnick, 2013). This species has been found to exhibit differences among populations in traits of epidemiological significance including foraging, oviposition, breeding preferences and resting patterns (Dickson et al., 2014; Gloria-Soria et al., 2016). In its native African range, it exists as two forms: the mainly domestic *Ae. aegypti aegypti* and sylvatic *Aedes aegypti formosus*. Sympatric occurrence of these forms has recently been suggested in many areas of Africa (Tabachnick and Powell, 1979; Powell, 2016; Gloria-Soria et al., 2016). Variability in competence to arboviruses among different geographic populations of *Ae. aegypti* is well documented (Black *et al.,* 2002; Chepkorir et al., 2014; Agha et al., 2017c). Much less is known whether differences in viral competence exist between the forms especially for endemic virus strains in Africa. Moreover, few studies have quantified their distribution in most areas (Brown et al., 2014; Gloria-Soria et al., 2016; Agha et al., 2019).

Recent outbreaks of dengue and chikungunya have been reported in parts of northeastern Kenya (Konongoi et al., 2016; Konongoi et al., 2018). We recently initiated an arboviral surveillance study focusing on flaviviruses in northern Kenya in two areas, West Pokot and Turkana Counties which border previous YF outbreak areas in Uganda, South Sudan and Ethiopia, with possibility of spill over into Kenya. Our previous findings showed variation in flavivirus human exposure risk in both areas. Higher rates of human exposure to dengue and Zika viruses in West Pokot were observed compared to Turkana County (Chepkorir et al., 2019). Such epidemiologic trend could reflect differences in the population structure of the local vector. In this study, we tested the hypothesis that population differences of the dengue vector, *Ae. aegypti* exists between the areas with the objectives to i) compare the genetic diversity and distribution of the forms of *Ae.*

aegypti from both areas and, ii) assess the vector competence of the species from both areas to transmit dengue-2 virus. We employed mitochondrial sequence data which have been valuable in characterising vector genetics and improve our understanding of vector-borne disease transmission (Tchouassi et al., 2014; Jaimes-Dueñez et al., 2015; Agha et al., 2019).

4.3 Methods

4.3.1 Ethical Considerations

Approval for this study was provided by Kenya Medical Research Institute (KEMRI) Centre for Virus Research Scientific Committee (CSC), the KEMRI Scientific and Ethics Review Unit (SERU), (under protocol number KEMRI-SERU 2787) and University of Pretoria.

4.3.2 Study site

This study was conducted in Kacheliba (West Pokot County) and Lokitoung (Turkana County) in Kenya. The counties border Uganda, South Sudan and Ethiopia (Figure 13) (Chepkorir *et al.*, 2019). The human populations in these counties are mainly nomadic pastoralists, however, currently there is increasing tendency for the people to migrate to urban centers within the counties, where drainage systems are poor and piped water supplies are erratic or nonexistent. This encourages water harvesting and storage, creating suitable environments for breeding of mosquitoes. There is also risk of viremic persons returning from neighboring countries, to initiate local transmission in Kenya with potential for more widespread activity.

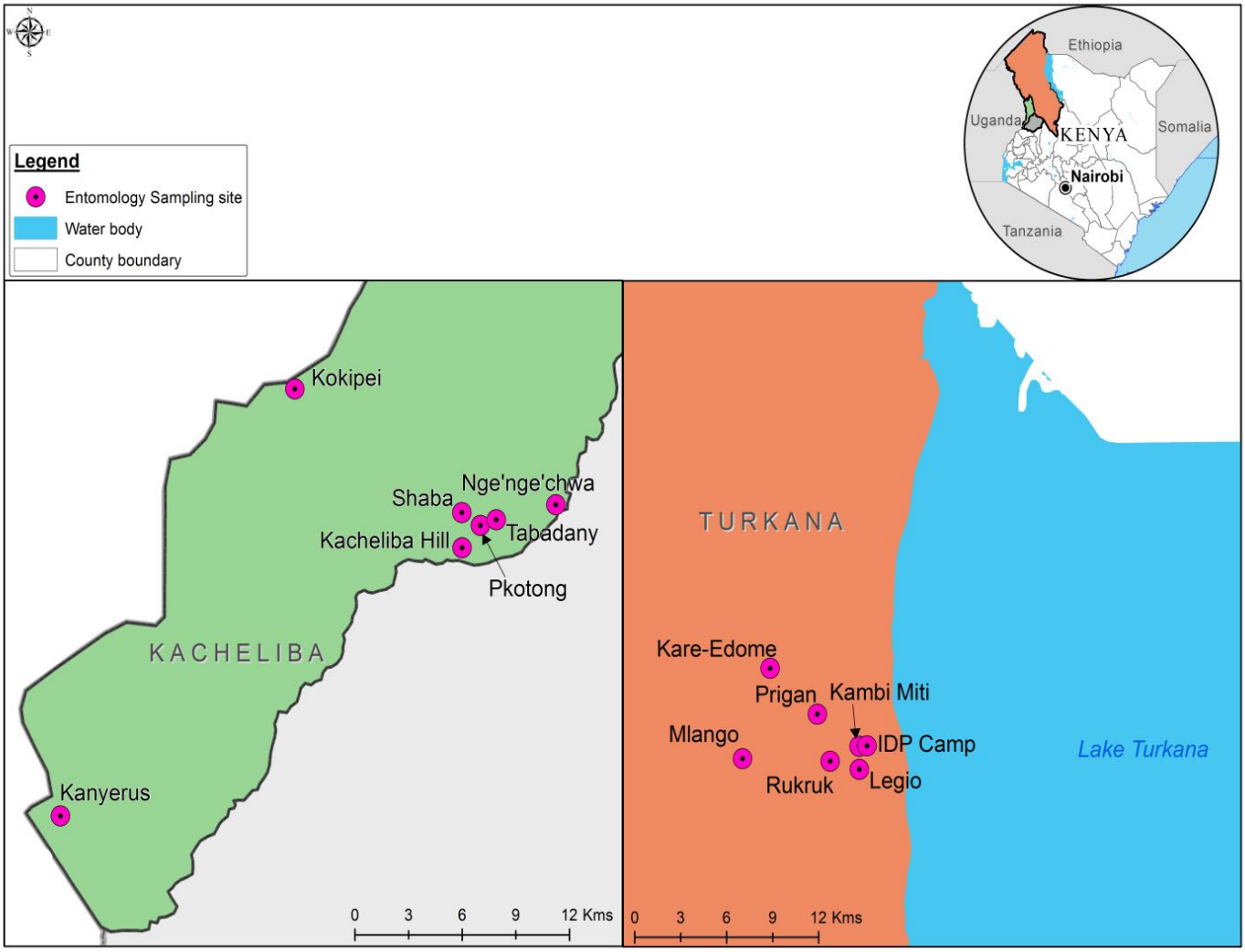

Figure 13: Map showing the entomological sampling sites in West Pokot and Turkana Counties, Kenya

4.3.3 Mosquito sampling and identification

Mosquitoes were collected from peridomestic areas in Kacheliba and Lokitoung. These included sites such as tree holes and caves which are habitats for rock dwelling rock hyraxes common in the areas. In addition, there were several rock pools that collect water creating ideal conditions for mosquito breeding after the rains. Adult and immature mosquitoes were surveyed during the

long rain seasons (May, 2015 and 2016) and the short rain season (December, 2015) in Kacheliba, West Pokot County. In Lokitoung, Turkana County, similar sampling was conducted in June 2017 and 2018 during the long rain season, in August, 2017 (dry season), December, 2017 (short rain season). Both areas are arid with erratic amounts of rainfall (Climate, 2019). Thus, a combination of trapping methods targeting adult and immatures were enforced to increase the chances of collection of *Ae. aegypti*. At each site, adult mosquito collection was done using CO_2-baited BG Sentinel traps set during the day (0600hrs to 1800hrs) and CO_2-baited CDC light traps set at 1800hrs and retrieved at 0600hrs (each ten traps per site). CO_2 was supplied in the form of dry ice and dispensed in Thermos flasks (~2L capacity). Resting mosquitoes in/outdoors, were collected using Procopack aspirators (BioQuip Products, Rancho Dominiguez, CA, USA). Larvae/pupae were collected using ladles, pipettes, and ovicups set to collect mosquito eggs. The collected eggs were hatched and like all the other immatures, reared to adults. Adult *Stegomyia* mosquitoes collected were transported in cages for rearing and infection in the Biosafety level 2 (BSL-2) insectary at KEMRI. Mosquitoes from the different sources were pooled for each site and used to set up colonies for use for molecular typing and vector competence studies (described below).

4.3.4 Mosquito rearing

Mosquitoes were reared in the KEMRI BSL-2 insectary, maintained at a temperature of 28°C and 80% relative humidity (RH), with a 12:12-hour (Light:Dark) photoperiod. As needed, several batches of eggs (F_0) from West Pokot and Turkana County sampling sites were dispensed in water on larval trays for hatching. The larvae were fed on tetramin fish food until they pupated. The pupae were collected daily and put in a cup containing water and then placed in a

1-gallon plastic cage with a netting material on top and allowed to develop into F_0 adult mosquitoes. The emerging adults were knocked down by placing them in a -20°C freezer for one minute, then morphologically identified under a dissecting microcope using taxonomic keys (Edwards, 1941; Huang, 2004), to ensure that only *Ae. aegypti* mosquitoes were used in the subsequent experiments. Identified *Ae. aegypti* mosquitoes were returned to their experimental cages, blood fed and provided with oviposition papers to lay F_1 eggs. The F_1 eggs were hatched and reared as decribed above (Agha et al., 2017c).

4.3.5 Molecular typing of *Ae. aegypti* from West Pokot and Turkana counties in Northern Kenya

4.3.5.1 DNA extraction and amplification

Individual mosquito (male/female) legs were separated from the rest of the body using a scalpel which was sterilized between successive decapitations. The legs were transferred into a sterile 1.5 ml microcentrifuge tube and triturated in 500µl of phosphate buffered saline (PBS). Genomic DNA was then extracted using the DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer's instructions. The DNA was used as the template in a standard polymerase chain reaction (PCR) to amplify the barcode region of Cytochrome Oxidase subunit 1 (*COI*) gene using published primers: COI FOR [5′-TGTAATTGTAACAGCTCATGCA-3′] and COI REV [5′-AATGATCATAGAAGGGCTGGAC-3′] (Paupy et al., 2012). PCR reaction was performed in a final reaction volume of 25µl containing Amplitaq Gold PCR master mix (Applied Biosystems), 0.5µM of both forward and reverse primers, RNAse/DNAse free water and genomic DNA template. The thermal profile for amplification was; enzyme activation at 94°C for 2 minutes, followed by 35 cycles of 94°C for 1 minute, 54°C for 30 seconds, 72°C for 1

minute and final elongation step at 72°C for 10 minutes. All amplification products (860 bp) were resolved in 1.5 % agarose gel electrophoresis.

4.3.5.2 DNA purification, sequencing and analysis

All PCR products were purified using a PCR purification kit (Wizard® SV Gel and PCR Clean-Up System from Promega) following the manufacturer's instructions. Bidirectional Sanger sequencing was outsourced to Inqaba (Pretoria, South Africa). The chromatograms of *COI* sequences were manually cleaned using ChromasPro Version 2.6.5. Contiguous sequences were generated using BioEdit software version 7.2.5. After trimming the *COI* sequences to remove ambiguous sites, final fragments of 726 bp were used in the genetic diversity and population genetic structure analysis. The sequences were aligned using the ClustalW option implemented in MEGA version 7 (Kumar et al., 2016). The aligned sequences were compared with the publicly available sequence data in GenBank using Basic Local Alignment Search Tool (BLASTn) to confirm species identification. The sequences generated in this study were deposited in GenBank under accession numbers MN171000 - MN171092.

4.3.5.3 Diversity, population structure and phylogeny

Diversity indices of the ingroup sequences, such as the number of polymorphic sites, haplotypes (h), haplotype diversity (Hd), nucleotide diversity (Pi), Tajima's D and Fu's Fs values were computed using DNA Sequence Polymorphism (DnaSP) version 6.12.03 (Rozas *et al.*, 2017). Significant negative values of these indices indicate a sudden population expansion whereas significant positive values indicate population subdivision or recent bottlenecks as described previously (Tchouassi et al., 2014).

Phylogenetic analysis was inferred using the Neighbor joining method based on the Tamura-Nei model with gamma distributed with Invariant sites (TN93+G+I) (Tamura and Nei, 1993) with MEGA 7.0. This was the best fit model of sequence evolution for the dataset as determined in MEGA 7.0 based on Akaike Information Criterion (AIC). We used GenBank sequence of *Aedes albopictus* (GenBank Accession No. MF148303) as outgroup. Bootstrap values for individual nodes were calculated for 5,000 replicates for the evaluation of tree robustness.

4.3.6 Vector competence studies

4.3.6.1 Dengue 2 virus amplification

Dengue virus serotype 2 (DEN-2) which was isolated from a patient's sample (Sample number: 008/01/2012) during the 2012 dengue outbreak in Mandera, Kenya, was used in the study. The virus was passaged in T-75cm^2 culture flask containing C6/36 cell lines (*Ae. albopictus* mosquito cell lines), grown in Dulbecco's modified eagles medium (DMEM), (GIBCO® Invitrogen corporation, Carlsbad, California), liquid (4.5 g/L D-glucose) without L-glutamine and sodium pyruvate, supplemented with 10% heat-inactivated fetal bovine serum (FBS), (Sigma-Aldrich, St. Louis, MO), 2% L-Glutamine (Sigma-Aldrich, St. Louis, MO), and 2% antibiotic/ antimycotic solution with 10,000 units penicillin, 10mg streptomycin and 25µg amphotericin B per ml (Sigma-Aldrich, St. Louis, MO) and incubated at 28°C in 5% CO_2 overnight. Confluent monolayers of C6/36 cells were inoculated with 600µl of the dengue virus supernatant isolate (Passage 2) and incubated for 1 hour with frequent agitation/rocking to allow for virus adsorption. The infected cells were maintained in DMEM supplemented with 2% FBS, 2% L-Glutamine and 2% antibiotic/ antimycotic, incubated at 28°C in 5% CO_2 and observed daily for cytopathic effect (CPE). On day 9 when CPE was observed to affect 80% of the monolayer, the

flask was frozen overnight at -80°C, thawed on wet ice, then clarified by centrifugation at 1500 revolutions per minute for 10 minutes and the supernatant harvested by aliquoting into 1.5ml cryovials. All the aliquots were stored at -80°C (Gubler et al., 1984).

4.3.6.2 Dengue 2 virus quantification

Quantification of dengue virus was performed by plaque assay. Ten fold serial dilutions of the amplified DENV was done and inoculated in 6 well plates containing confluent Vero cell monolayers (CCL-81^{TM}) as previously described (Gargan *et al.*,1983). This was grown in Minimum Essential Medium Eagle (MEM), (Sigma-Aldrich, St. Louis, MO) with Earle's salts and reduced $NaHCO_3$, supplemented with 10% heat-inactivated fetal bovine serum (FBS), (Sigma-Aldrich, St. Louis, MO), 2% L-Glutamine (Sigma-Aldrich, St. Louis, MO), and 2% antibiotic/ antimycotic solution with 10,000 units penicillin, 10mg streptomycin and 25µg amphotericin B per ml (Sigma-Aldrich, St. Louis, MO) and incubated at 37°C in 5% CO_2 overnight. Each well was inoculated with 100µl of virus dilution, incubated for 1 hour with frequent agitation/rocking to allow adsorption. The infected cells were maintained using 2.5% methylcellulose mixed with 2X MEM (Sigma) and incubated at 37°C with 5% CO_2 for 9 days then fixed for 1 hour with 10% formalin, stained for 2 hours with 0.5% crystal violet (Sigma) diluted in absolute ethanol, and the plaques counted and calculated to quantify the virus using the formula (Gargan *et al.*,1983):

$$\frac{\text{Number of plaques}}{\text{d} \times \text{V}} = \text{pfu/ml}$$

where d is the dilution factor and V is the volume of diluted virus added to the well.

4.3.6.3 Mosquito infection with Dengue 2 virus

An infectious blood meal was prepared by mixing DEN-2 virus stock (Virus titer of $10^{5.08\text{-}5.3}$ plaque-forming units (PFU)/ml) and defibrinated sheep blood, in a ratio of 1:1. The virus/blood mixture was put in membrane feeders (Higgs and Beaty, 1996), and maintained using the Hemotek system which employs an electric heating element to maintain the temperature of the blood meal at 35°C±1°C (Cosgrove et al., 1994). Four-day-old adult female mosquitoes were allowed to feed on the infectious blood meal through mouse skin for 1hour. After feeding, fully engorged mosquitoes were selected and put in secured cages, where they were maintained on 8% glucose for 7 to 21 days. Mortality was monitored in the cages by removing and counting dead mosquitoes daily (Cosgrove et al., 1994).

4.3.6.4 Test for Infection and dissemination of *Ae. aegypti* to Dengue 2 virus

After every 7 days up to day 21 of incubation, 30% of live exposed mosquitoes were randomly selected and each dissected to separate the body and legs (Table 9). Each mosquito body was placed separately in 1.5ml eppendorf tubes containing 500µl of homogenization media (HM), consisting of MEM, supplemented with 15% FBS, 2% L-Glutamine, and 2% antibiotic/ antimycotic. The individual bodies were homogenized, and the supernatant diluted in 10-fold serial dilutions up to 10^{-3}. The dilutions were inoculated in confluent Vero cell lines in 12-well plates, grown in MEM, supplemented with 10% FBS, 2% L-Glutamine and 2% antibiotic/ antimycotic. The infected cell monolayers were then overlaid with 2.5% methylcellulose (Sigma) supplemented with 2% FBS, 2% L-Glutamine and 2% antibiotic/ antimycotic solution and incubated at 37°C in 5% CO_2. On day 9, plates were fixed for 1 hour with 10% formalin, and stained for 2 hours with 0.5% crystal violet, washed on running tap water and dried overnight.

The plaques formed were counted and calculated as described previously. For each positive body, corresponding legs were homogenized and their infection status determined as previously described. Plaques were counted and calculated using the formula described by Gargan (1983) for each mosquito legs, to determine the dissemination rates. If the virus was detected in the mosquito's body, but not in the legs, the mosquito was considered to have a non-disseminated infection limited to the midgut (Turell *et al.*, 1984).

Table 9: The number of *Ae. aegypti* mosquitoes sampled after every 7 days post infection

Strain	**Total no. sampled**	**Days post Infection**		
		7	**14**	**21**
West Pokot	243	80	80	83
Turkana	241	81	79	81

4.4 Results

4.4.1 Genetic variability

A total of 90 *Ae. aegypti* mosquitoes were analysed for genetic variability. This represented 59 specimens from West Pokot and 31 from Turkana. Specimens from West Pokot were representative of collections made across all seasons mainly sampled as immatures (Table 10). On the other hand, specimens used for Turkana were limited to collections of few immatures only. Adult collections in all the seasons yielded no mosquitoes; thus, the fewer specimens from Turkana.

Table 10: Information on *Aedes aegypti* collections from the two sites, used in the study

Strains	Habitat type	Breeding sites	Date of collection	Collection method	Developmental Stage collected	Sample size
West Pokot	Peridomestic	Artificial containers Sisal axils Rock pool Tree holes	May 2015 December 2015 May 2016	CDC Light trap	Adults	4
				BG sentinel traps	Adults	16
				Prokopack	Adults	3
				Ladles/Pipettes	Pupae/Larvae	33
				Ovicups	Eggs	3
						59
Turkana	Peridomestic	Artificial containers	June 2017 August 2017 December 2017 June 2018	Ladles/Pipettes	Pupae/Larvae	**31**
Total						**90**

West Pokot county had the highest number of haplotypes (h= 21) compared to Turkana county (h= 6) with corresponding haplotype diversity, Hd of 0.828 for West Pokot and 0.570 for Turkana (Table 11). The nucleotide diversity (Pi), was lower in Turkana (0.003) compared to West Pokot (0.0090). The results for neutrality tests show that both Tajima's D and Fu's Fs values were not significant in any of the two sites ($p > 0.100$) (Table 11).

Table 11: Genetic diversity indices, neutrality test values for *Aedes aegypti* samples from West Pokot and Turkana Counties

Strains	S	H	Hd(±SD)	Pi(±SD)	D	Fs
West Pokot	20	21	0.828±0.041	0.0090±0.00054	-0.16006	-6.763
Turkana	10	6	0.570±0.97	0.003±0.00063	-0.19979	0.951
Two sites combined	22	25	0.874±0.021	0.00828±0.00052	-0.39594	-9.218

S no. of polymorphic sites, *h* number of haplotypes, *Hd* haplotype diversity, *Pi* nucleotide diversity, *SD* Standard deviation, *D* Tajima's D, *Fs* Fu's Fs

COI gene phylogeny showed that *Ae. aegypti* resolves as two lineages. One of the lineages had all the samples from Turkana, a proportion of samples from West Pokot and clustered with the sylvatic formosus form of *Ae. aegypti* (GenBank accession no. AY056597, MH410195, MH410191, MH410205) (Figure 14). The second lineage within which the domestic form of *Ae. aegypti* (GenBank accession nos. MH410187, MH410196, MH410194, MH410207) clustered, and exclusively had samples from West Pokot. Thus, only samples from West Pokot were represented in both lineages.

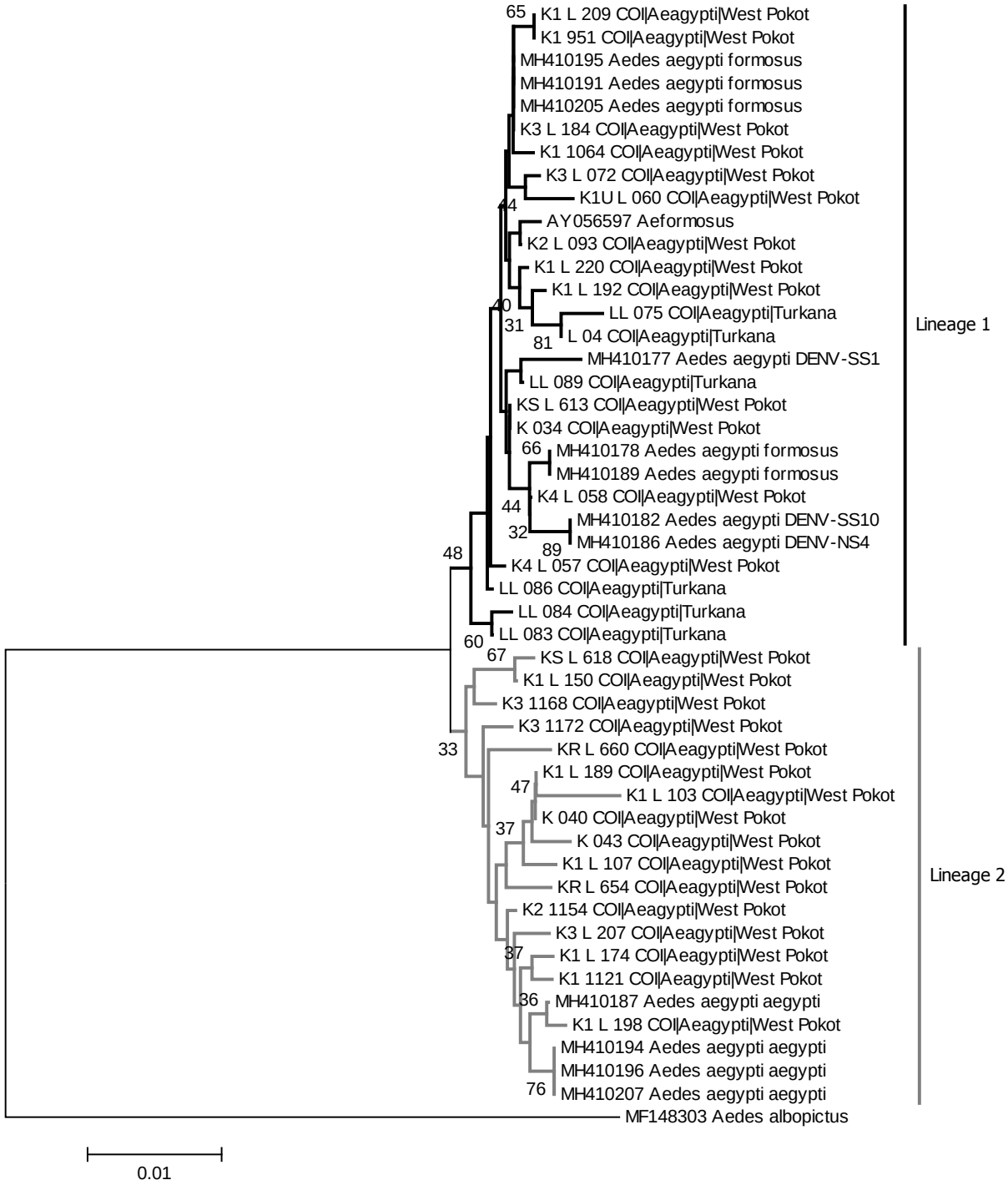

Figure 14: A Neighbor joining phylogeny based on COI gene sequences of *Ae. aegypti* mosquitoes collected from Turkana and West Pokot Counties, Kenya. The tree is rooted with a sequence of *Ae. albopictus* (Accession no. MF148303). The tree was generated based on Tamura-Nei model with gamma distributed with Invariant sites (TN93+G+I) (Tamura and Nei, 1993). The tip labels of the individual sequences are annotated based on the origin location of the sequences. Evolutionary analyses were conducted in MEGA7 with 5000 bootstraps (Kumar *et al.*, 2016).

4.4.2 Vector competence

Dengue-2 virus susceptibility was assessed in a total of 484 *Ae. aegypti* female mosquitoes (West Pokot, n=243; Turkana, n=241). Overall dengue-2 infection rate of 9.1% (n=44) was observed. However, the infection rate was higher for *Ae. aegypti* from West Pokot than Turkana (13.6% vs 4.6%; P=0.0001). Figure 14 presents the percentage of infected mosquitoes at day 7, 14 and 21 post-infection with the highest infection recorded on day 14 post-infection. There was no disseminated infection.

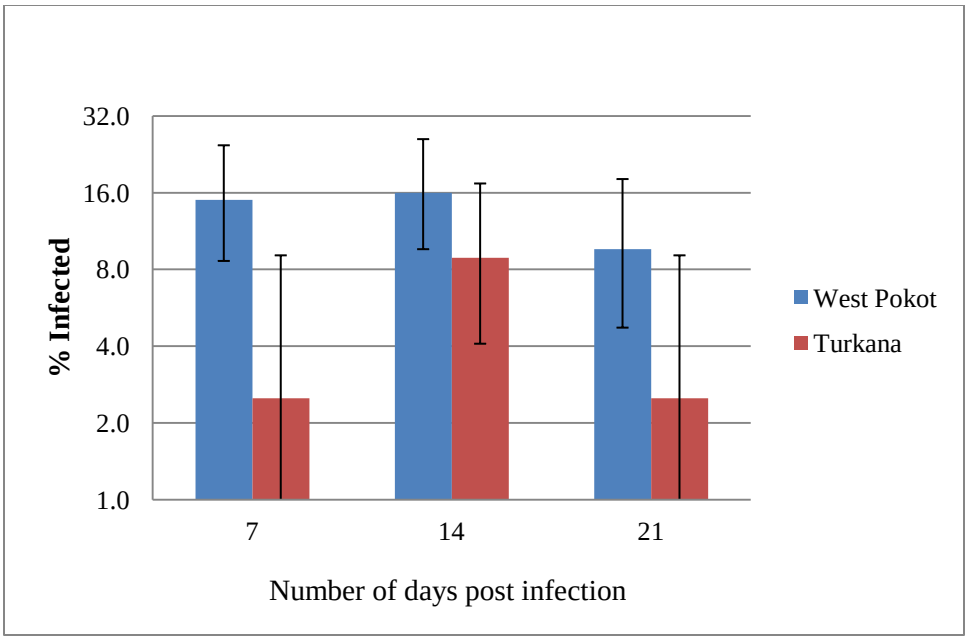

Figure 15: Proportion of West Pokot and Turkana *Ae. aegypti* population infected at day 7, 14 and 21 post infection with dengue- 2 virus. The error bars indicate Agresti-Coull 95% confidence intervals.

4.5 Discussion

Phylogenetic analysis of *Ae. aegypti* suggests differences in representation of mitochondrial lineages from West Pokot and Turkana counties. The two mitochondrial lineages seem to mirror the two distinct genetic clusters corresponding to the forms widely known in literature (Brown et al., 2011; Powell, 2016; Gloria-Soria et al., 2016). A recent study has corroborated these forms as distinct genetics entities through a worldwide study using microsatellite markers (Gloria-Soria et al., 2016). Some of the West Pokot samples grouped with mitochondrial lineage same as *formosus* form. This may suggest that *Ae. aegypti* from this site is predominantly sylvatic in nature. Likewise, specimens from West Pokot appear to have a representation of both forms

dominated by the domestic form. Our data show that samples from West Pokot clustered in both mitochondrial lineages in sympatry. The findings are consistent with those of previous studies carried out on mosquito population from Coastal Kenya, where the two subspecies were found to coexist (Lounibos, 2003; Brown *et al.*, 2011; Gloria-Soria *et al.*, 2016; Agha *et al.*, 2019).

Adaptation of mosquitoes to different environment is dependent on the availability of breeding sites and human activities (Powell & Tabacknick, 2013; Patz et al., 2000). Even though *Ae. aegypti* strains have evolved from their sylvatic foci to adapt and proliferate in containers in urban areas (Powell & Tabacknick, 2013), most of the mosquitoes sampled from West Pokot county were collected as immatures from natural breeding sites (Tree holes, rock pools and sisal axils). Those from Turkana County were collected from artificial containers this could be as a result of high temperatures in Turkana county that led to the drying up of the natural breeding sites. A study on host blood feeding preferences of *Ae. aegypti* mosquitoes from West Pokot county displayed zoophilic tendency mainly feeding on wild and domestic animals (hyrax and goat), however a few had fed on human (Chepkorir et al., 2018). This shows a unique adaptation of *Ae. aegypti aegypti* present in the region. Even with several sampling trips to Turkana County, there was no blood fed adult mosquito collected and therefore could not determine the Turkana mosquito population host blood feeding preference.

An important epidemiologic question is whether genetic variants of *Ae. aegypti* impact differentially in pathogen transmission. Vector competence largely depends on the genetics of a given vector and virus (Lounibos & Kramer, 2016). The vector competence findings in this study showed that the mosquito population from West Pokot County was three times more susceptible

to dengue 2 virus compared to the Turkana county mosquito population. This suggests possible midgut infection barriers in the Turkana mosquito population. Studies have shown that populations of *Ae. aegypti* vary considerably in efficiency of transmitting disease causing viruses and that *Ae. aegypti aegypti* is more susceptible to dengue 2 virus compared to *Ae. aegypti formosus* (Black *et al.,* 2002; Sylla *et al.,* 2009). Intriguing, there was no evidence of dissemination or transmission for both populations suggesting the presence of a midgut escape barrier (Turell et al., 1984). Specimens analysed for genetic characterisations from West Pokot were drawn randomly from same pool of samples. Thus, there is reason to believe that samples from this site used for infection studies are representative of both mitochondrial lineages. Overall, the data suggest relative incompetent vectors in the absence of dissemination or transmission abilities (Lounibos & Kramer, 2016). Nonetheless, very incompetent vectors have been found capable of initiating and sustaining arbovirus outbreaks in the presence of high population density (Miller et al., 1989; Grubaugh et al., 2017). The variation in mitochondrial lineage between the sites may impact in the observed difference in infection rates.

Whether the high prevalence of Zika and presence of dengue virus antibodies in serum samples collected from West Pokot, compared to those from Turkana County (Chepkorir et al., 2019) is related to the presence of *Ae. aegypti aegypti* in the region, is not clear. Studies involving vector competence of *Ae. aegypti aegypti* to transmit Zika virus and more on the different serotypes of DENV will unravel the much needed information on disease transmission in the region.

We did not observe disseminated infection and transmission in the two vector populations. The observation could be inherent in the populations although other methodological issues are worth considering. First, this may relate to the history of virus stocks used for blood meal preparation i.e., whether used as frozen or freshly harvested to infect mosquitoes. A study by Richards

(Richards et al., 2007) found that the rates of infection and dissemination for mosquitoes fed with blood meals containing DENV-2, were significantly lower ($P < 0.05$) in mosquitoes exposed to frozen–thawed versus freshly collected virus. The reason for diminished infectivity in fresh compared to frozen–thawed virus is unknown (Richards et al., 2007). This is unlikely to be the case, as both the frozen and freshly grown DENV-2 were used in this study, and we found no difference in infection nor dissemination rates. Second, the passage history of the virus can affect this observation (Ciota et al., 2007). The DEN-2 virus (008/01/2012) used in this study was isolated from human serum during the year 2012 dengue outbreak in Mandera, Northeastern Kenya (Konongoi et al., 2016). The virus was first passaged in C6/36 cells and later amplified in Vero cells in preparation for infection studies. The DENV-2 virus used in this study was at passage 3, and given that the experimental design was uniformly applied across the mosquito population, there is little evidence this factor could have generated the observed differences in infection rates. Third, the titre of virus used is an important determinant of mosquito infectivity, and subsequently transmission, is the orally available dose (Kuno and Chang, 2005). The virus titre used in this study was $10^{5.08\text{-}5.3}$ plaque-forming units (PFU)/ml, which is consistent with virus titres used in previous vector competence studies of *Ae. aegypti* mosquito populations that produced disseminated infection (Chepkorir *et al.*, 2014).

Our study had some limitations. The results presented here on the genetic variability of the different mosquito populations were based on only one targeted gene. While highlighting the differences in the genetic structure of *Ae. aegypti* between the sites, additional studies could benefit from use of additional markers and sampling for a longer duration and incorporating a larger sample size. Previous studies have however found association between mitochondrial

variants and mosquito infection states (Pham Thi *et al.*, 2017). This data therefore could not be used to give details on the evolutionary history of these mosquito populations.

In conclusion, this study highlights the presence of the two forms of *Ae. aegypti* (*Ae. aegypti aegypti* and *Ae. aegypti formosus*) in the two border counties. The data revealed that the susceptibility of the two *Ae. aegypti* populations to dengue 2 virus varied significantly. The data contribute, in part, to our understanding of the epidemiological trends observed in the previous chapters.

Chapter 5

5.1 Concluding remarks

Yellow fever and dengue are diseases that are difficult to diagnose and confirm, because their symptoms can be mild and mistaken for other infections like malaria and typhoid. These are diseases that mostly occur in some of the most resource-poor settings globally causing strain to the public health resources. Both YFV and DENV are members of the family *Flaviviridae*, genus *Flavivirus* and are transmitted by same vectors and maintained in same reservoirs. Assessment of risk for these diseases requires a co-ordinate approach, for example; surveillance data reflect patterns of endemicity and emergence of infections in areas that previously had not been studied, but the data may not reflect the actual disease burden. Therefore, to successfully tackle the growing threat of these Flaviviral diseases, in part, depends on strengthening the evidence base on which control planning decisions and their impact are evaluated. Knowledge on risk factors such as mosquito density, host feeding pattern, level of human exposure, vector genetics and

vector competence when evaluated collectively, will certainly inform risk of transmission of these Flaviviral diseases. In this study, we combined field base survey and laboratory based analysis to assess the risk of transmission of YFV and DENV in West Pokot and Turkana counties, bordering countries that have recently reported outbreaks of YF and dengue (Uganda, South Sudan and Ethiopia).

Chapter 2 provides a useful baseline and first report on the mosquito fauna inhabiting the ecology of this study area in Kenya. The presence of important *Stegomyia* species, which are known vectors of YFV and DENV, in Kacheliba, West Pokot County, suggests the level of risk for transmission of these viruses and potential for possible outbreaks of YF and DEN in this area. However, as observed on figure 8, the *Stegomyia* species from these sites displayed a strong zoophagic tendency, mainly feeding on wild and domestic animals (hyrax and goat), an attribute that would reduce potential for disease transmission to human. Thus there is need for further bionomic and surveillance studies on mosquitoes including determination of vector competence of the *Stegomyia* species detected. Transmission potential of a pathogen is defined by vectorial capacity which depends on a range of factors and not only human blood feeding and vector abundance. An important factor includes vector survival which is the most sensitive determinant of vectorial capacity and needs to be studied. A vector must live sufficiently long to complete extrinsic incubation of the virus and thereafter is infected and able to transmit for the remainder of its life. Future studies could benefit from high throughput approaches that can sensitively detect circulating viruses even before human exposure. This should target all mosquito species of ecologic importance and not specific ones.

In chapter 3, the findings demonstrated human exposure to infections with *Flaviviruses* in West Pokot and Turkana counties of Kenya. The evidence of circulation of other important

Flaviviruses like West Nile and Zika virus in this population especially in the 13-19 age group shows a recent circulation that had gone undetected in the human population. The findings suggest active transmission of WNV in Turkana and ZIKV transmission in West Pokot County. The variable exposure risk observed between the two areas could be related to differences in climate and geography between them. Therefore, it would be worthwhile for future studies to assess the local seasonal mosquito fauna composition and abundance trends, and investigate their vector competence to transmit ZIKV, in order to shed more light on the high prevalence observed. Evidence of YF seropositivity may partly be due to previous vaccination, and if so, there is a potential risk of YF transmission if vaccination is not enhanced to improve herd immunity to protective level of 80%, considering proximity to an outbreak area across the border and the presence of potential vectors. One Health approach through active surveillance to integrate the myriad of factors involved in modulating human exposure risk is crucial to developing a decision support tool for accurate risk assessment.

In chapter 4, the data suggest differential distribution of mitochondrial lineages between West Pokot and Turkana counties, and this could explain differences in susceptibilities between populations of the species to dengue-2 virus. Studies on vector competence for this virus i.e., the ability for a given vector to acquire and subsequently transmit a pathogen, are important component of risk assessment to improve our understanding of patterns or spread of re-emerging viruses such as dengue. More detailed studies should be conducted using other dengue serotypes, to estimate other variables of the vectorial capacity for a comprehensive quantification of arboviral risk of assessment between the sites. The study also, confirmed the presence of both *Ae. aegypti aegypti* and *Ae. aegypti formosus* in West Pokot and Turkana counties in Northern Kenya. The difference in their susceptibility to dengue 2 virus varied significantly. However, to

conclusively identify the genetic basis of adaptation, it will be necessary to sample more intensively over several seasons, to allow for more powerful tests of selection and ultimately link these differences to phenotypic changes.

While the findings of this research thesis suggest, low risk of YF and dengue in West Pokot and Turkana counties, it underscores the need for continued monitoring of the border region to enable appropriate and timely intervention by public health authorities. Overall, the findings provide needed evidence of human exposure to these important flaviviruses for early warning and, for action towards disease prevention plans and the activation of preparedness strategies to counter potential threats posed by these viruses. Additionally, these findings will guide health officials to consider these virus infections as they manage febrile cases that do not respond to regular treatment for diseases like malaria, typhoid, etc, which are usually diagnosed clinically where laboratory capacity is limited. It is our recommendation to the Ministry of Health to provide and encourage point of care diagnostics for these viruses.

Chapter 6

6.1 REFERENCES

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Appendices

Appendix 1: Multiple sequence alignment of the haplotypes used to generate the phylogeny

KC690897_Aedes_albopictus_COI	ATAATTGGAGGATTTGGAAACTGACTAGTACCCTTAATACTAGGAGCCCC	50
AF390098_Aedes_aegypti	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
AY056597_Aeformosus	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
Aedes_aegypti_MF194022	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
LL_089_COI|Aeagypti|Turkana	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
LL_086_COI|Aeagypti|Turkana	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
LL_084_COI|Aeagypti|Turkana	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
LL_083_COI|Aeagypti|Turkana	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
LL_075_COI|Aeagypti|Turkana	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
L_04_COI|Aeagypti|Turkana	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
KS_L_618_COI|Aeagypti|West_Pok	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
KS_L_613_COI|Aeagypti|West_Pok	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
KS_L_606_COI|Aeagypti|West_Pok	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
KR_L_660_COI|Aeagypti|West_Pok	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
KR_L_654_COI|Aeagypti|West_Pok	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
K_043_COI|Aeagypti|West_Pokot	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
K_040_COI|Aeagypti|West_Pokot	ATAATTGGAGGATTTGGAAATTGATTAGTTCCCTTAATATTAGGAGCCCC	50
K_034_COI|Aeagypti|West_Pokot	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
K4_L_058_COI|Aeagypti|West_Pok	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
K4_L_057_COI|Aeagypti|West_Pok	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
K3_L_207_COI|Aeagypti|West_Pok	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
K3_L_184_COI|Aeagypti|West_Pok	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
K3_L_072_COI|Aeagypti|West_Pok	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
K3_1172_COI|Aeagypti|West_Poko	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
K3_1168_COI|Aeagypti|West_Poko	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
K2_L_093_COI|Aeagypti|West_Pok	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
K2_1154_COI|Aeagypti|West_Poko	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
K1U_L_060_COI|Aeagypti|West_Po	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
K1_L_220_COI|Aeagypti|West_Pok	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50

K1_L_209_COI|Aeagypti|West_Pok	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
K1_L_198_COI|Aeagypti|West_Pok	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
K1_L_192_COI|Aeagypti|West_Pok	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
K1_L_189_COI|Aeagypti|West_Pok	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
K1_L_174_COI|Aeagypti|West_Pok	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
K1_L_150_COI|Aeagypti|West_Pok	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
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K1_L_103_COI|Aeagypti|West_Pok	ATAATTGGAGGATTTGGAAATTGATTAGTTCCCTTAATATTAGGAGCCCC	50
K1_1121_COI|Aeagypti|West_Poko	ATAATTGGAGGATTTGGAAATTGATTAGTTCCCTTAATATTAGGAGCCCC	50
K1_1064_COI|Aeagypti|West_Poko	ATAATTGGAGGATTCGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50
K1_951_COI|Aeagypti|West_Pokot	ATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATATTAGGAGCCCC	50

KC690897_Aedes_albopictus_COI	TGATATAGCTTTTCCTCGAATAAATAATATAAGTTTTTGAATATTACCCC	100
AF390098_Aedes_aegypti	TGATATAGCTTTCCCTCGAATGAATAATATAAGTTTTTGAATACTACCTC	100
AY056597_Aeformosus	TGATATAGCCTTTCCTCGAATAAATAATATAAGTTTTTGAATACTACCTC	100
Aedes_aegypti_MF194022	TGATATAGCTTTCCCTCGAATGAATAATATAAGTTTTTGAATACTACCTC	100
LL_089_COI|Aeagypti|Turkana	TGATATAGCCTTTCCTCGAATAAATAATATAAGTTTTTGAATACTACCTC	100
LL_086_COI|Aeagypti|Turkana	TGATATAGCTTTTCCTCGAATAAATAATATAAGTTTTTGAATACTACCTC	100
LL_084_COI|Aeagypti|Turkana	TGATATAGCCTTTCCTCGAATAAATAATATAAGTTTTTGAATACTACCTC	100
LL_083_COI|Aeagypti|Turkana	TGATATAGCCTTTCCTCGAATAAATAATATAAGTTTTTGAATACTACCTC	100
LL_075_COI|Aeagypti|Turkana	TGATATAGCCTTTCCTCGAATAAATAATATAAGTTTTTGAATACTACCTC	100
L_04_COI|Aeagypti|Turkana	TGATATAGCCTTTCCTCGAATAAATAATATAAGTTTTTGAATACTACCTC	100
KS_L_618_COI|Aeagypti|West_Pok	TGATATAGCTTTCCCTCGAATGAATAATATAAGTTTTTGAATACTACCTC	100
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KS_L_606_COI|Aeagypti|West_Pok	TGATATAGCTTTCCCTCGAATGAATAATATAAGTTTTTGAATACTACCTC	100
KR_L_660_COI|Aeagypti|West_Pok	TGATATAGCTTTCCCTCGAATAAATAATATAAGTTTTTGAATACTACCTC	100
KR_L_654_COI|Aeagypti|West_Pok	TGATATAGCTTTCCCTCGAATAAATAATATAAGTTTTTGAATACTACCTC	100
K_043_COI|Aeagypti|West_Pokot	TGATATAGCTTTCCCTCGAATGAATAATATAAGTTTTTGAATACTACCTC	100
K_040_COI|Aeagypti|West_Pokot	TGATATAGCTTTCCCTCGAATAAATAATATAAGTTTTTGAATACTACCTC	100
K_034_COI|Aeagypti|West_Pokot	TGATATAGCTTTTCCTCGAATAAATAATATAAGTTTTTGAATACTACCTC	100
K4_L_058_COI|Aeagypti|West_Pok	TGATATAGCCTTTCCTCGAATAAATAATATAAGTTTTTGAATACTACCTC	100
K4_L_057_COI|Aeagypti|West_Pok	TGATATAGCCTTTCCTCGAATAAATAATATAAGTTTTTGAATACTACCTC	100
K3_L_207_COI|Aeagypti|West_Pok	TGATATAGCTTTCCCTCGAATGAATAATATAAGTTTTTGAATACTACCTC	100
K3_L_184_COI|Aeagypti|West_Pok	TGATATAGCTTTTCCTCGAATAAATAATATAAGTTTTTGAATACTACCTC	100
K3_L_072_COI|Aeagypti|West_Pok	TGATATAGCCTTTCCTCGAATAAATAATATAAGTTTTTGAATACTACCTC	100

K3_1172_COI|Aeagypti|West_Poko	TGATATAGCTTTCCCTCGAATAAATAATATAAGTTTTTGAATACTACCTC	100
K3_1168_COI|Aeagypti|West_Poko	TGATATAGCTTTCCCTCGAATAAATAATATAAGTTTTTGAATACTACCTC	100
K2_L_093_COI|Aeagypti|West_Pok	TGATATAGCCTTTCCTCGAATAAATAATATAAGTTTTTGAATACTACCTC	100
K2_1154_COI|Aeagypti|West_Poko	TGATATAGCTTTCCCTCGAATGAATAATATAAGTTTTTGAATACTACCTC	100
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K1_951_COI|Aeagypti|West_Pokot	TGATATAGCCTTTCCTCGAATAAATAATATAAGTTTTTGAATACTACCTC	100

KC690897_Aedes_albopictus_COI	CCTCTTTAACACTGCTGCTTTCTAGTTCTATAGTAGAAAACGGAGCTGGA	150
AF390098_Aedes_aegypti	CTTCATTGACTCTTCTATTATCAAGCTCAATAGTAGAAAATGGGGCAGGA	150
AY056597_Aeformosus	CTTCATTGACTCTTCTATTATCAAGCTCAATAGTAGAAAATGGGGCAGGA	150
Aedes_aegypti_MF194022	CTTCATTGACTCTTCTATTATCAAGCTCAATAGTAGAAAATGGGGCAGGA	150
LL_089_COI|Aeagypti|Turkana	CTTCATTGACTCTTCTATTATCAAGCTCAATAGTAGAAAATGGAGCAGGA	150
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KS_L_613_COI|Aeagypti|West_Pok	CTTCATTGACTCTTCTATTATCAAGCTCAATAGTAGAAAATGGAGCAGGA	150
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K_043_COI|Aeagypti|West_Pokot	CTTCATTGACTCTTCTATTATCAAGCTCAATAGTAGAAAATGGAGCAGGA	150
K_040_COI|Aeagypti|West_Pokot	CTTCATTGACTCTTCTATTATCAAGCTCAATAGTAGAAAATGGAGCAGGA	150

K_034_COI|Aeagypti|West_Pokot	CTTCATTGACTCTTCTATTATCAAGCTCAATAGTAGAAAATGGAGCAGGA	150
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K1U_L_060_COI|Aeagypti|West_Po	CTTCATTGACTCTTCTATTATCAAGCTCAATAGTAGAAAATGGGGCAGGA	150
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K1_L_209_COI|Aeagypti|West_Pok	CTTCATTGACTCTTCTATTATCAAGCTCAATAGTAGAAAATGGGGCAGGA	150
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K1_L_192_COI|Aeagypti|West_Pok	CTTCATTGACTCTTCTATTATCAAGCTCAATAGTAGAAAATGGAGCAGGA	150
K1_L_189_COI|Aeagypti|West_Pok	CTTCATTGACTCTTCTATTATCAAGCTCAATAGTAGAAAATGGAGCAGGA	150
K1_L_174_COI|Aeagypti|West_Pok	CTTCATTGACTCTTCTATTATCAAGCTCAATAGTAGAAAATGGAGCAGGA	150
K1_L_150_COI|Aeagypti|West_Pok	CTTCATTGACTCTTCTATTATCAAGCTCAATAGTAGAAAATGGAGCAGGA	150
K1_L_107_COI|Aeagypti|West_Pok	CTTCATTGACTCTTCTATTATCAAGCTCAATAGTAGAAAATGGGGCAGGA	150
K1_L_103_COI|Aeagypti|West_Pok	CTTCATTGACTCTTCTATTATCAAGCTCAATAGTAGAAAATGGAGCAGGA	150
K1_1121_COI|Aeagypti|West_Poko	CTTCATTGACTCTTCTATTATCAAGCTCAATAGTAGAAAATGGAGCAGGA	150
K1_1064_COI|Aeagypti|West_Poko	CTTCATTGACTCTTCTATTATCAAGCTCAATAGTAGAAAATGGGGCAGGA	150
K1_951_COI|Aeagypti|West_Pokot	CTTCATTGACTCTTCTATTATCAAGCTCAATAGTAGAAAATGGGGCAGGA	150
KC690897_Aedes_albopictus_COI	ACAGGGTGAACGGTTTATCCTCCTCTTTCTTCTGGAACAGCTCATGCTGG	200
AF390098_Aedes_aegypti	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
AY056597_Aeformosus	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
Aedes_aegypti_MF194022	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
LL_089_COI|Aeagypti|Turkana	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
LL_086_COI|Aeagypti|Turkana	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
LL_084_COI|Aeagypti|Turkana	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
LL_083_COI|Aeagypti|Turkana	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
LL_075_COI|Aeagypti|Turkana	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
L_04_COI|Aeagypti|Turkana	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
KS_L_618_COI|Aeagypti|West_Pok	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200

KS_L_613_COI|Aeagypti|West_Pok	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
KS_L_606_COI|Aeagypti|West_Pok	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
KR_L_660_COI|Aeagypti|West_Pok	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
KR_L_654_COI|Aeagypti|West_Pok	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K_043_COI|Aeagypti|West_Pokot	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K_040_COI|Aeagypti|West_Pokot	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K_034_COI|Aeagypti|West_Pokot	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K4_L_058_COI|Aeagypti|West_Pok	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K4_L_057_COI|Aeagypti|West_Pok	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K3_L_207_COI|Aeagypti|West_Pok	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K3_L_184_COI|Aeagypti|West_Pok	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K3_L_072_COI|Aeagypti|West_Pok	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K3_1172_COI|Aeagypti|West_Poko	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K3_1168_COI|Aeagypti|West_Poko	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K2_L_093_COI|Aeagypti|West_Pok	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K2_1154_COI|Aeagypti|West_Poko	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K1U_L_060_COI|Aeagypti|West_Po	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K1_L_220_COI|Aeagypti|West_Pok	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K1_L_209_COI|Aeagypti|West_Pok	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K1_L_198_COI|Aeagypti|West_Pok	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K1_L_192_COI|Aeagypti|West_Pok	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K1_L_189_COI|Aeagypti|West_Pok	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K1_L_174_COI|Aeagypti|West_Pok	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K1_L_150_COI|Aeagypti|West_Pok	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K1_L_107_COI|Aeagypti|West_Pok	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K1_L_103_COI|Aeagypti|West_Pok	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K1_1121_COI|Aeagypti|West_Poko	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K1_1064_COI|Aeagypti|West_Poko	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200
K1_951_COI|Aeagypti|West_Pokot	ACTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAACAGCTCATGCTGG	200

KC690897_Aedes_albopictus_COI	GGCTTCAGTTGATTTAGCAATTTTTTCTTTACATTTAGCGGGAATCTCAT	250
AF390098_Aedes_aegypti	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
AY056597_Aeformosus	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
Aedes_aegypti_MF194022	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
LL_089_COI|Aeagypti|Turkana	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250

LL_086_COI|Aeagypti|Turkana	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
LL_084_COI|Aeagypti|Turkana	GGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
LL_083_COI|Aeagypti|Turkana	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
LL_075_COI|Aeagypti|Turkana	AGCTTCTGTTGATCCAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
L_04_COI|Aeagypti|Turkana	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
KS_L_618_COI|Aeagypti|West_Pok	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
KS_L_613_COI|Aeagypti|West_Pok	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
KS_L_606_COI|Aeagypti|West_Pok	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
KR_L_660_COI|Aeagypti|West_Pok	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
KR_L_654_COI|Aeagypti|West_Pok	GGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K_043_COI|Aeagypti|West_Pokot	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K_040_COI|Aeagypti|West_Pokot	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K_034_COI|Aeagypti|West_Pokot	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K4_L_058_COI|Aeagypti|West_Pok	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K4_L_057_COI|Aeagypti|West_Pok	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K3_L_207_COI|Aeagypti|West_Pok	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K3_L_184_COI|Aeagypti|West_Pok	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K3_L_072_COI|Aeagypti|West_Pok	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K3_1172_COI|Aeagypti|West_Poko	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K3_1168_COI|Aeagypti|West_Poko	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K2_L_093_COI|Aeagypti|West_Pok	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K2_1154_COI|Aeagypti|West_Poko	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K1U_L_060_COI|Aeagypti|West_Po	GGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K1_L_220_COI|Aeagypti|West_Pok	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K1_L_209_COI|Aeagypti|West_Pok	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K1_L_198_COI|Aeagypti|West_Pok	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K1_L_192_COI|Aeagypti|West_Pok	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K1_L_189_COI|Aeagypti|West_Pok	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K1_L_174_COI|Aeagypti|West_Pok	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K1_L_150_COI|Aeagypti|West_Pok	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K1_L_107_COI|Aeagypti|West_Pok	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K1_L_103_COI|Aeagypti|West_Pok	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K1_1121_COI|Aeagypti|West_Poko	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K1_1064_COI|Aeagypti|West_Poko	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250
K1_951_COI|Aeagypti|West_Pokot	AGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCTGGAATTTCCT	250

KC690897_Aedes_albopictus_COI	CTATTTTAGGAGCAGTAAATTTTATTACAACTGTAATTAATATACGATCA	300
AF390098_Aedes_aegypti	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTGATTAATATGCGATCG	300
AY056597_Aeformosus	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
Aedes_aegypti_MF194022	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTGATTAATATGTGATCG	300
LL_089_COI|Aeagypti|Turkana	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
LL_086_COI|Aeagypti|Turkana	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
LL_084_COI|Aeagypti|Turkana	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
LL_083_COI|Aeagypti|Turkana	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
LL_075_COI|Aeagypti|Turkana	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
L_04_COI|Aeagypti|Turkana	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
KS_L_618_COI|Aeagypti|West_Pok	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
KS_L_613_COI|Aeagypti|West_Pok	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
KS_L_606_COI|Aeagypti|West_Pok	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
KR_L_660_COI|Aeagypti|West_Pok	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
KR_L_654_COI|Aeagypti|West_Pok	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
K_043_COI|Aeagypti|West_Pokot	CAATTTTAGGAGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
K_040_COI|Aeagypti|West_Pokot	CAATTTTAGGAGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
K_034_COI|Aeagypti|West_Pokot	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
K4_L_058_COI|Aeagypti|West_Pok	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
K4_L_057_COI|Aeagypti|West_Pok	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
K3_L_207_COI|Aeagypti|West_Pok	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
K3_L_184_COI|Aeagypti|West_Pok	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
K3_L_072_COI|Aeagypti|West_Pok	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
K3_1172_COI|Aeagypti|West_Poko	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
K3_1168_COI|Aeagypti|West_Poko	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
K2_L_093_COI|Aeagypti|West_Pok	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
K2_1154_COI|Aeagypti|West_Poko	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
K1U_L_060_COI|Aeagypti|West_Po	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
K1_L_220_COI|Aeagypti|West_Pok	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
K1_L_209_COI|Aeagypti|West_Pok	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
K1_L_198_COI|Aeagypti|West_Pok	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
K1_L_192_COI|Aeagypti|West_Pok	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
K1_L_189_COI|Aeagypti|West_Pok	CAATTTTAGGAGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
K1_L_174_COI|Aeagypti|West_Pok	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCA	300

K1_L_150_COI|Aeagypti|West_Pok	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
K1_L_107_COI|Aeagypti|West_Pok	CAATTTTAGGAGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
K1_L_103_COI|Aeagypti|West_Pok	CAATTTTAGGAGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
K1_1121_COI|Aeagypti|West_Poko	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
K1_1064_COI|Aeagypti|West_Poko	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
K1_951_COI|Aeagypti|West_Pokot	CAATTTTAGGGGCAGTAAATTTTATTACAACTGTAATTAATATACGATCG	300
KC690897_Aedes_albopictus_COI	GCTGGTATTACTCTTGATCGACTACCTTTATTTGTGTGATCAGTAGTAAT	350
AF390098_Aedes_aegypti	TCAGGGATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
AY056597_Aeformosus	TCAGGAATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
Aedes_aegypti_MF194022	TCAGGGATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
LL_089_COI|Aeagypti|Turkana	TCAGGAATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
LL_086_COI|Aeagypti|Turkana	TCAGGAATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
LL_084_COI|Aeagypti|Turkana	TCAGGAATTACTTTAGATCGACTACCTTTATTTGTTTGATCTGTAGTTAT	350
LL_083_COI|Aeagypti|Turkana	TCAGGAATTACTTTAGATCGACTACCTTTATTTGTTTGATCTGTAGTTAT	350
LL_075_COI|Aeagypti|Turkana	TCAGGAATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
L_04_COI|Aeagypti|Turkana	TCAGGAATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
KS_L_618_COI|Aeagypti|West_Pok	TCAGGAATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
KS_L_613_COI|Aeagypti|West_Pok	TCAGGAATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
KS_L_606_COI|Aeagypti|West_Pok	TCAGGGATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
KR_L_660_COI|Aeagypti|West_Pok	TCAGGGATTACTTTAGATCGACTACCTTTATTTGTTTGATCTGTAGTTAT	350
KR_L_654_COI|Aeagypti|West_Pok	TCAGGGATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
K_043_COI|Aeagypti|West_Pokot	TCAGGGATTACTTTAGATCGACTACCCTTATTTGTATGATCTGTAGTTAT	350
K_040_COI|Aeagypti|West_Pokot	TCAGGGATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
K_034_COI|Aeagypti|West_Pokot	TCAGGAATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
K4_L_058_COI|Aeagypti|West_Pok	TCAGGAATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
K4_L_057_COI|Aeagypti|West_Pok	TCAGGAATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
K3_L_207_COI|Aeagypti|West_Pok	TCAGGGATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
K3_L_184_COI|Aeagypti|West_Pok	TCAGGAATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
K3_L_072_COI|Aeagypti|West_Pok	TCAGGAATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
K3_1172_COI|Aeagypti|West_Poko	TCAGGGATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
K3_1168_COI|Aeagypti|West_Poko	TCAGGAATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
K2_L_093_COI|Aeagypti|West_Pok	TCAGGAATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
K2_1154_COI|Aeagypti|West_Poko	TCAGGGATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
K1U_L_060_COI|Aeagypti|West_Po	TCAGGAATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350

K1_L_220_COI|Aeagypti|West_Pok	TCAGGAATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
K1_L_209_COI|Aeagypti|West_Pok	TCAGGAATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
K1_L_198_COI|Aeagypti|West_Pok	TCAGGGATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
K1_L_192_COI|Aeagypti|West_Pok	TCAGGAATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
K1_L_189_COI|Aeagypti|West_Pok	TCAGGGATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
K1_L_174_COI|Aeagypti|West_Pok	TCAGGGATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
K1_L_150_COI|Aeagypti|West_Pok	TCAGGAATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
K1_L_107_COI|Aeagypti|West_Pok	TCAGGGATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
K1_L_103_COI|Aeagypti|West_Pok	TCAGGGATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
K1_1121_COI|Aeagypti|West_Poko	TCAGGGATTACTTTAGATCGACTACCCTTATTTGTATGATCTGTAGTTAT	350
K1_1064_COI|Aeagypti|West_Poko	TCAGGAATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350
K1_951_COI|Aeagypti|West_Pokot	TCAGGAATTACTTTAGATCGACTACCCTTATTTGTTTGATCTGTAGTTAT	350

KC690897_Aedes_albopictus_COI	TACAGCTATTTTATTACTTCTTTCTCTACCCGTATTAGCCGGAGCTATTA	400
AF390098_Aedes_aegypti	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
AY056597_Aeformosus	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGGGCTATTA	400
Aedes_aegypti_MF194022	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
LL_089_COI|Aeagypti|Turkana	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
LL_086_COI|Aeagypti|Turkana	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
LL_084_COI|Aeagypti|Turkana	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
LL_083_COI|Aeagypti|Turkana	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
LL_075_COI|Aeagypti|Turkana	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
L_04_COI|Aeagypti|Turkana	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
KS_L_618_COI|Aeagypti|West_Pok	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
KS_L_613_COI|Aeagypti|West_Pok	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
KS_L_606_COI|Aeagypti|West_Pok	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
KR_L_660_COI|Aeagypti|West_Pok	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
KR_L_654_COI|Aeagypti|West_Pok	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
K_043_COI|Aeagypti|West_Pokot	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
K_040_COI|Aeagypti|West_Pokot	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
K_034_COI|Aeagypti|West_Pokot	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
K4_L_058_COI|Aeagypti|West_Pok	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
K4_L_057_COI|Aeagypti|West_Pok	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
K3_L_207_COI|Aeagypti|West_Pok	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
K3_L_184_COI|Aeagypti|West_Pok	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400

K3_L_072_COI|Aeagypti|West_Pok	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
K3_1172_COI|Aeagypti|West_Poko	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
K3_1168_COI|Aeagypti|West_Poko	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
K2_L_093_COI|Aeagypti|West_Pok	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
K2_1154_COI|Aeagypti|West_Poko	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
K1U_L_060_COI|Aeagypti|West_Po	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
K1_L_220_COI|Aeagypti|West_Pok	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
K1_L_209_COI|Aeagypti|West_Pok	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
K1_L_198_COI|Aeagypti|West_Pok	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
K1_L_192_COI|Aeagypti|West_Pok	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
K1_L_189_COI|Aeagypti|West_Pok	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
K1_L_174_COI|Aeagypti|West_Pok	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
K1_L_150_COI|Aeagypti|West_Pok	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
K1_L_107_COI|Aeagypti|West_Pok	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
K1_L_103_COI|Aeagypti|West_Pok	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
K1_1121_COI|Aeagypti|West_Poko	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400
K1_1064_COI|Aeagypti|West_Poko	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCCGGAGCTATTA	400
K1_951_COI|Aeagypti|West_Pokot	TACAGCTATCTTATTACTTCTTTCTCTTCCTGTTTTAGCTGGAGCTATTA	400

KC690897_Aedes_albopictus_COI	CTATATTATTAACAGACCGAAATTTAAATACATCTTTTTTTGATCCAATT	450
AF390098_Aedes_aegypti	CTATATTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
AY056597_Aeformosus	CTATGTTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
Aedes_aegypti_MF194022	CTATATTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
LL_089_COI|Aeagypti|Turkana	CTATGTTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
LL_086_COI|Aeagypti|Turkana	CTATATTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
LL_084_COI|Aeagypti|Turkana	CTATATTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
LL_083_COI|Aeagypti|Turkana	CTATATTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
LL_075_COI|Aeagypti|Turkana	CTATGTTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
L_04_COI|Aeagypti|Turkana	CTATGTTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
KS_L_618_COI|Aeagypti|West_Pok	CTATATTATTAACAGACCGAAACTTAAATACATCTTTCTTTGACCCAATC	450
KS_L_613_COI|Aeagypti|West_Pok	CTATGTTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
KS_L_606_COI|Aeagypti|West_Pok	CTATATTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
KR_L_660_COI|Aeagypti|West_Pok	CTATATTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATT	450
KR_L_654_COI|Aeagypti|West_Pok	CTATATTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
K_043_COI|Aeagypti|West_Pokot	CTATATTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450

K_040_COI|Aeagypti|West_Pokot	CTATATTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
K_034_COI|Aeagypti|West_Pokot	CTATGTTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
K4_L_058_COI|Aeagypti|West_Pok	CTATGTTATTAACAGACCGAAACTTAAATACATCTTTCTTTGACCCAATC	450
K4_L_057_COI|Aeagypti|West_Pok	CTATATTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
K3_L_207_COI|Aeagypti|West_Pok	CTATATTATTAACAGACCGAAACTTAAATACATCTTTCTTTGACCCAATC	450
K3_L_184_COI|Aeagypti|West_Pok	CTATGTTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
K3_L_072_COI|Aeagypti|West_Pok	CTATGTTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
K3_1172_COI|Aeagypti|West_Poko	CTATATTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
K3_1168_COI|Aeagypti|West_Poko	CTATATTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
K2_L_093_COI|Aeagypti|West_Pok	CTATGTTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
K2_1154_COI|Aeagypti|West_Poko	CTATATTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
K1U_L_060_COI|Aeagypti|West_Po	CTATGTTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATT	450
K1_L_220_COI|Aeagypti|West_Pok	CTATGTTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
K1_L_209_COI|Aeagypti|West_Pok	CTATGTTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
K1_L_198_COI|Aeagypti|West_Pok	CTATATTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
K1_L_192_COI|Aeagypti|West_Pok	CTATGTTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
K1_L_189_COI|Aeagypti|West_Pok	CTATATTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
K1_L_174_COI|Aeagypti|West_Pok	CTATATTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
K1_L_150_COI|Aeagypti|West_Pok	CTATATTATTAACAGACCGAAACTTAAATACATCTTTCTTTGACCCAATC	450
K1_L_107_COI|Aeagypti|West_Pok	CTATATTATTAACAGCCCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
K1_L_103_COI|Aeagypti|West_Pok	CTATATTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
K1_1121_COI|Aeagypti|West_Poko	CTATATTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
K1_1064_COI|Aeagypti|West_Poko	CTATGTTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
K1_951_COI|Aeagypti|West_Pokot	CTATGTTATTAACAGACCGAAACTTAAATACATCTTTCTTTGATCCAATC	450
KC690897_Aedes_albopictus_COI	GGAGGAGGAGACCCTATTTTATATCAACATTTATTTTGATTTTTTGGTCA	500
AF390098_Aedes_aegypti	GGAGGGGGAGACCCTATTTTATACCAACACTTATTTTGATTCTTTGGACA	500
AY056597_Aeformosus	GGAGGAGGAGACCCTATTTTATACCAACACTTATTCTGATTCTTTGGACA	500
Aedes_aegypti_MF194022	GGAGGGGGAGACCCTATTTTATACCAACACTTATTTTGATTCTTTGGACA	500
LL_089_COI|Aeagypti|Turkana	GGAGGAGGAGACCCTATTTTATACCAACACTTATTCTGATTCTTTGGACA	500
LL_086_COI|Aeagypti|Turkana	GGAGGAGGAGATCCTATTTTATACCAACACTTATTCTGATTCTTTGGACA	500
LL_084_COI|Aeagypti|Turkana	GGAGGAGGAGACCCTATTTTATACCAACACTTATTCTGATTCTTTGGACA	500
LL_083_COI|Aeagypti|Turkana	GGAGGAGGAGACCCTATTTTATACCAACACTTATTCTGATTCTTTGGACA	500
LL_075_COI|Aeagypti|Turkana	GGAGGAGGAGACCCTATTTTATACCAACACTTATTCTGATTCTTTGGACA	500
L_04_COI|Aeagypti|Turkana	GGAGGAGGAGACCCTATTTTATACCAACACTTATTCTGATTCTTTGGACA	500

KS_L_618_COI|Aeagypti|West_Pok	GGAGGGGGAGACCCTATTTTATACCAACACTTATTTTGATTCTTTGGGCA	500
KS_L_613_COI|Aeagypti|West_Pok	GGAGGAGGAGATCCTATTTTATACCAACACTTATTCTGATTCTTTGGACA	500
KS_L_606_COI|Aeagypti|West_Pok	GGAGGGGGAGACCCTATTTTATACCAACACTTATTTTGATTCTTTGGACA	500
KR_L_660_COI|Aeagypti|West_Pok	GGAGGAGGAGACCCTATTTTATACCAACACTTATTTTGATTCTTTGGACA	500
KR_L_654_COI|Aeagypti|West_Pok	GGAGGGGGAGACCCTATTTTATACCAACACTTATTTTGATTCTTTGGACA	500
K_043_COI|Aeagypti|West_Pokot	GGAGGGGGAGACCCTATTTTATACCAACACTTATTTTGATTCTTTGGACA	500
K_040_COI|Aeagypti|West_Pokot	GGAGGGGGAGACCCTATTTTATACCAACACTTATTTTGATTCTTTGGACA	500
K_034_COI|Aeagypti|West_Pokot	GGAGGAGGAGATCCTATTTTATACCAACACTTATTCTGATTCTTTGGACA	500
K4_L_058_COI|Aeagypti|West_Pok	GGAGGAGGAGATCCTATTTTATACCAACACTTATTCTGATTCTTTGGACA	500
K4_L_057_COI|Aeagypti|West_Pok	GGAGGAGGAGATCCTATTTTATACCAACACTTATTCTGATTCTTTGGACA	500
K3_L_207_COI|Aeagypti|West_Pok	GGAGGAGGAGACCCTATTTTATACCAACACTTATTTTGATTCTTTGGGCA	500
K3_L_184_COI|Aeagypti|West_Pok	GGAGGAGGAGATCCTATTTTATACCAACACTTATTCTGATTCTTTGGACA	500
K3_L_072_COI|Aeagypti|West_Pok	GGAGGAGGAGATCCTATTTTATACCAACACTTATTCTGATTCTTTGGACA	500
K3_1172_COI|Aeagypti|West_Poko	GGAGGAGGAGACCCTATTTTATACCAACACTTATTTTGATTCTTTGGGCA	500
K3_1168_COI|Aeagypti|West_Poko	GGAGGGGGAGACCCTATTTTATACCAACACTTATTTTGATTCTTTGGACA	500
K2_L_093_COI|Aeagypti|West_Pok	GGAGGAGGAGACCCTATTTTATACCAACACTTATTCTGATTCTTTGGACA	500
K2_1154_COI|Aeagypti|West_Poko	GGAGGAGGAGACCCTATTTTATACCAACACTTATTTTGATTCTTTGGACA	500
K1U_L_060_COI|Aeagypti|West_Po	GGAGGAGGAGATCCTATTTTATACCAACACTTATTCTGATTCTTTGGACA	500
K1_L_220_COI|Aeagypti|West_Pok	GGAGGAGGAGATCCTATTTTATACCAACACTTATTCTGATTCTTTGGACA	500
K1_L_209_COI|Aeagypti|West_Pok	GGAGGAGGAGATCCTATTTTATACCAACACTTATTCTGATTCTTTGGACA	500
K1_L_198_COI|Aeagypti|West_Pok	GGAGGGGGAGACCCTATTTTATACCAACACTTATTTTGATTCTTTGGGCA	500
K1_L_192_COI|Aeagypti|West_Pok	GGAGGAGGAGACCCTATTTTATACCAACACTTATTCTGATTCTTTGGACA	500
K1_L_189_COI|Aeagypti|West_Pok	GGAGGGGGAGACCCTATTTTATACCAACACTTATTTTGATTCTTTGGACA	500
K1_L_174_COI|Aeagypti|West_Pok	GGAGGGGGAGACCCTATTTTATACCAACACTTATTTTGATTCTTTGGGCA	500
K1_L_150_COI|Aeagypti|West_Pok	GGAGGGGGAGACCCTATTTTATACCAACACTTATTTTGATTCTTTGGGCA	500
K1_L_107_COI|Aeagypti|West_Pok	GGAGGGGGAGACCCTATTTTATACCAACACTTATTTTGATTCTTTGGACA	500
K1_L_103_COI|Aeagypti|West_Pok	GGAGGGGGAGACCCTATTTTATACCAACACTTATTTTTATTCTTTGGACA	500
K1_1121_COI|Aeagypti|West_Poko	GGAGGGGGAGACCCTATTTTATACCAACACTTATTTTGATTCTTTGGGCA	500
K1_1064_COI|Aeagypti|West_Poko	GGAGGAGGAGATCCTATTTTATACCAACACTTATTCTGATTCTTTGGACA	500
K1_951_COI|Aeagypti|West_Pokot	GGAGGAGGAGATCCTATTTTATACCAACACTTATTCTGATTCTTTGGACA	500

KC690897_Aedes_albopictus_COI	TCCAGAAGTTTATATTTTAATTCTGCCAGGATTTGGAATAATTTCTCATA	550
AF390098_Aedes_aegypti	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
AY056597_Aeformosus	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
Aedes_aegypti_MF194022	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550

LL_089_COI|Aeagypti|Turkana	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
LL_086_COI|Aeagypti|Turkana	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
LL_084_COI|Aeagypti|Turkana	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
LL_083_COI|Aeagypti|Turkana	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
LL_075_COI|Aeagypti|Turkana	CCCAGAAGTTTATATTTTAATTTTACCTGGATTTGGAATAATTTCTCATA	550
L_04_COI|Aeagypti|Turkana	CCCAGAAGTTTATATTTTAATTTTACCTGGATTTGGAATAATTTCTCATA	550
KS_L_618_COI|Aeagypti|West_Pok	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
KS_L_613_COI|Aeagypti|West_Pok	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
KS_L_606_COI|Aeagypti|West_Pok	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
KR_L_660_COI|Aeagypti|West_Pok	CCCAGAAGTTTATATTTTAATTTTACCTGGATTTGGAATAATTTCTCATA	550
KR_L_654_COI|Aeagypti|West_Pok	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
K_043_COI|Aeagypti|West_Pokot	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
K_040_COI|Aeagypti|West_Pokot	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
K_034_COI|Aeagypti|West_Pokot	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
K4_L_058_COI|Aeagypti|West_Pok	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
K4_L_057_COI|Aeagypti|West_Pok	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
K3_L_207_COI|Aeagypti|West_Pok	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
K3_L_184_COI|Aeagypti|West_Pok	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
K3_L_072_COI|Aeagypti|West_Pok	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
K3_1172_COI|Aeagypti|West_Poko	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
K3_1168_COI|Aeagypti|West_Poko	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
K2_L_093_COI|Aeagypti|West_Pok	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
K2_1154_COI|Aeagypti|West_Poko	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
K1U_L_060_COI|Aeagypti|West_Po	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
K1_L_220_COI|Aeagypti|West_Pok	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
K1_L_209_COI|Aeagypti|West_Pok	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
K1_L_198_COI|Aeagypti|West_Pok	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
K1_L_192_COI|Aeagypti|West_Pok	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
K1_L_189_COI|Aeagypti|West_Pok	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
K1_L_174_COI|Aeagypti|West_Pok	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
K1_L_150_COI|Aeagypti|West_Pok	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
K1_L_107_COI|Aeagypti|West_Pok	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
K1_L_103_COI|Aeagypti|West_Pok	GGCAGAGGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
K1_1121_COI|Aeagypti|West_Poko	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550
K1_1064_COI|Aeagypti|West_Poko	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550

K1_951_COI|Aeagypti|West_Pokot	CCCAGAAGTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCTCATA	550

KC690897_Aedes_albopictus_COI	TTATTACACAAGAAAGAGGAAAAAAGGAAACTTTTGGTACTTTAGGAATA	600
AF390098_Aedes_aegypti	TTATTACTCAAGAAAGCGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
AY056597_Aeformosus	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
Aedes_aegypti_MF194022	TTATTACTCAAGAAAGCGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
LL_089_COI|Aeagypti|Turkana	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
LL_086_COI|Aeagypti|Turkana	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
LL_084_COI|Aeagypti|Turkana	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
LL_083_COI|Aeagypti|Turkana	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
LL_075_COI|Aeagypti|Turkana	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
L_04_COI|Aeagypti|Turkana	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
KS_L_618_COI|Aeagypti|West_Pok	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
KS_L_613_COI|Aeagypti|West_Pok	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
KS_L_606_COI|Aeagypti|West_Pok	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
KR_L_660_COI|Aeagypti|West_Pok	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
KR_L_654_COI|Aeagypti|West_Pok	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
K_043_COI|Aeagypti|West_Pokot	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
K_040_COI|Aeagypti|West_Pokot	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
K_034_COI|Aeagypti|West_Pokot	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
K4_L_058_COI|Aeagypti|West_Pok	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
K4_L_057_COI|Aeagypti|West_Pok	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
K3_L_207_COI|Aeagypti|West_Pok	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
K3_L_184_COI|Aeagypti|West_Pok	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
K3_L_072_COI|Aeagypti|West_Pok	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
K3_1172_COI|Aeagypti|West_Poko	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
K3_1168_COI|Aeagypti|West_Poko	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
K2_L_093_COI|Aeagypti|West_Pok	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
K2_1154_COI|Aeagypti|West_Poko	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
K1U_L_060_COI|Aeagypti|West_Po	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
K1_L_220_COI|Aeagypti|West_Pok	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
K1_L_209_COI|Aeagypti|West_Pok	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
K1_L_198_COI|Aeagypti|West_Pok	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
K1_L_192_COI|Aeagypti|West_Pok	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
K1_L_189_COI|Aeagypti|West_Pok	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600

K1_L_174_COI|Aeagypti|West_Pok	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
K1_L_150_COI|Aeagypti|West_Pok	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
K1_L_107_COI|Aeagypti|West_Pok	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
K1_L_103_COI|Aeagypti|West_Pok	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
K1_1121_COI|Aeagypti|West_Poko	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
K1_1064_COI|Aeagypti|West_Poko	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
K1_951_COI|Aeagypti|West_Pokot	TTATTACTCAAGAAAGTGGAAAAAAGGAAACATTTGGAACTTTAGGAATA	600
KC690897_Aedes_albopictus_COI	ATTTATGCTATATTAACAATTGGCTTATTAGGATTTATTGTATGAGCCCA	650
AF390098_Aedes_aegypti	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
AY056597_Aeformosus	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
Aedes_aegypti_MF194022	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
LL_089_COI|Aeagypti|Turkana	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
LL_086_COI|Aeagypti|Turkana	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
LL_084_COI|Aeagypti|Turkana	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
LL_083_COI|Aeagypti|Turkana	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
LL_075_COI|Aeagypti|Turkana	ATTTATGCTATATTAACAATTGGATTATTAGGATTTATTGTTTGAGCTCA	650
L_04_COI|Aeagypti|Turkana	ATTTATGCTATATTAACAATTGGATTATTAGGATTTATTGTTTGAGCTCA	650
KS_L_618_COI|Aeagypti|West_Pok	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
KS_L_613_COI|Aeagypti|West_Pok	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
KS_L_606_COI|Aeagypti|West_Pok	ATTTATGCTATATTAACAATTGGATTATTAGGATTTATTGTTTGAGCTCA	650
KR_L_660_COI|Aeagypti|West_Pok	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
KR_L_654_COI|Aeagypti|West_Pok	ATTTATGCTATATTAACAATTGGATTATTGGGGTTTATTGTTTGAGCTCA	650
K_043_COI|Aeagypti|West_Pokot	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
K_040_COI|Aeagypti|West_Pokot	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
K_034_COI|Aeagypti|West_Pokot	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
K4_L_058_COI|Aeagypti|West_Pok	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
K4_L_057_COI|Aeagypti|West_Pok	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
K3_L_207_COI|Aeagypti|West_Pok	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
K3_L_184_COI|Aeagypti|West_Pok	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
K3_L_072_COI|Aeagypti|West_Pok	ATTTATGCTATATTAACAATTGGATTATTGGGGTTTATTGTTTGAGCTCA	650
K3_1172_COI|Aeagypti|West_Poko	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
K3_1168_COI|Aeagypti|West_Poko	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
K2_L_093_COI|Aeagypti|West_Pok	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
K2_1154_COI|Aeagypti|West_Poko	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650

K1U_L_060_COI|Aeagypti|West_Po	ATTTATGCTATATTAACAATTGGATTATTGGGGTTTATTGTTTGAGCTCA	650
K1_L_220_COI|Aeagypti|West_Pok	ATTTATGCTATATTAACAATTGGATTATTAGGATTTATTGTTTGAGCTCA	650
K1_L_209_COI|Aeagypti|West_Pok	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
K1_L_198_COI|Aeagypti|West_Pok	ATTTATGCTATATTAACAATTGGATTATTAGGATTTATTGTTTGAGCTCA	650
K1_L_192_COI|Aeagypti|West_Pok	ATTTATGCTATATTAACAATTGGATTATTAGGATTTATTGTTTGAGCTCA	650
K1_L_189_COI|Aeagypti|West_Pok	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
K1_L_174_COI|Aeagypti|West_Pok	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
K1_L_150_COI|Aeagypti|West_Pok	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
K1_L_107_COI|Aeagypti|West_Pok	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
K1_L_103_COI|Aeagypti|West_Pok	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
K1_1121_COI|Aeagypti|West_Poko	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
K1_1064_COI|Aeagypti|West_Poko	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650
K1_951_COI|Aeagypti|West_Pokot	ATTTATGCTATATTAACAATTGGATTATTGGGATTTATTGTTTGAGCTCA	650

KC690897_Aedes_albopictus_COI	TCATATATTCACAGTTGGTATAGATGTTGATACTCGAGCTTATTTTACGT	700
AF390098_Aedes_aegypti	TCATATATTTACAGTAGGTATAGACGTAGATACTCGAGCTTATTTTACTT	700
AY056597_Aeformosus	TCATATATTTACAGTAGGTATAGACGTAGATACTCGAGCTTATTTTACTT	700
Aedes_aegypti_MF194022	TCATATATTTACAGTAGGTATAGACGTAGATACTCGAGCTTATTTTACTT	700
LL_089_COI|Aeagypti|Turkana	TCATATATTTACAGTAGGTATAGACGTAGATACTCGAGCTTATTTTACTT	700
LL_086_COI|Aeagypti|Turkana	TCATATATTTACAGTAGGTATAGACGTAGATACTCGAGCTTATTTTACTT	700
LL_084_COI|Aeagypti|Turkana	TCATATATTTACAGTAGGTATAGACGTAGATACTCGAGCTTATTTTACTT	700
LL_083_COI|Aeagypti|Turkana	TCATATATTTACAGTAGGTATAGACGTAGATACTCGAGCTTATTTTACTT	700
LL_075_COI|Aeagypti|Turkana	TCATATATTTACAGTAGGTATAGACGTAGATACTCGAGCTTATTTTACTT	700
L_04_COI|Aeagypti|Turkana	TCATATATTTACAGTAGGTATAGACGTAGATACTCGAGCTTATTTTACTT	700
KS_L_618_COI|Aeagypti|West_Pok	TCATATATTTACAGTAGGTATAGACGTAGATACTCGAGCTTATTTTACTT	700
KS_L_613_COI|Aeagypti|West_Pok	TCATATATTTACAGTAGGTATAGACGTAGATACTCGAGCTTATTTTACTT	700
KS_L_606_COI|Aeagypti|West_Pok	TCATATATTTACAGTAGGTATAGACGTAGATACTCGAGCTTATTTTACTT	700
KR_L_660_COI|Aeagypti|West_Pok	TCATATATTTACAGTAGGTATAGATGTAGATACTCGAGCTTATTTTACTT	700
KR_L_654_COI|Aeagypti|West_Pok	TCATATATTTACAGTAGGTATAGATGTAGATACTCGAGCTTATTTTACTT	700
K_043_COI|Aeagypti|West_Pokot	TCATATATTTACAGTAGGTATAGATGTAGATACTCGAGCTTATTTTACTT	700
K_040_COI|Aeagypti|West_Pokot	TCATATATTTACAGTAGGTATAGATGTAGATACTCGAGCTTATTTTACTT	700
K_034_COI|Aeagypti|West_Pokot	TCATATATTTACAGTAGGTATAGACGTAGATACTCGAGCTTATTTTACTT	700
K4_L_058_COI|Aeagypti|West_Pok	TCATATATTTACAGTAGGTATAGACGTAGATACTCGAGCTTATTTTACTT	700
K4_L_057_COI|Aeagypti|West_Pok	TCATATATTTACAGTAGGTATAGACGTAGATACTCGAGCTTATTTTACTT	700
K3_L_207_COI|Aeagypti|West_Pok	TCATATATTTACAGTAGGTATAGATGTAGATACTCGAGCTTATTTTACTT	700

K3_L_184_COI|Aeagypti|West_Pok	TCATATATTTACAGTAGGTATAGACGTAGATACTCGAGCTTATTTTACTT	700
K3_L_072_COI|Aeagypti|West_Pok	TCATATATTTACAGTAGGTATAGACGTAGATACTCGAGCTTATTTTACTT	700
K3_1172_COI|Aeagypti|West_Poko	TCATATATTTACAGTAGGTATAGATGTAGATACTCGAGCTTATTTTACTT	700
K3_1168_COI|Aeagypti|West_Poko	TCATATATTTACAGTAGGTATAGATGTAGATACTCGAGCTTATTTTACTT	700
K2_L_093_COI|Aeagypti|West_Pok	TCATATATTTACAGTAGGTATAGACGTAGATACTCGAGCTTATTTTACTT	700
K2_1154_COI|Aeagypti|West_Poko	TCATATATTTACAGTAGGTATAGATGTAGATACTCGAGCTTATTTTACTT	700
K1U_L_060_COI|Aeagypti|West_Po	TCATATATTTACAGTAGGTATAGACGTAGATACTCGAGCTTATTTTACTT	700
K1_L_220_COI|Aeagypti|West_Pok	TCATATATTTACAGTAGGTATAGACGTAGATACTCGAGCTTATTTTACTT	700
K1_L_209_COI|Aeagypti|West_Pok	TCATATATTTACAGTAGGTATAGATGTAGATACTCGAGCTTATTTTACTT	700
K1_L_198_COI|Aeagypti|West_Pok	TCATATATTTACAGTAGGTATAGATGTAGATACTCGAGCTTATTTTACTT	700
K1_L_192_COI|Aeagypti|West_Pok	TCATATATTTACAGTAGGTATAGACGTAGATACTCGAGCTTATTTTACTT	700
K1_L_189_COI|Aeagypti|West_Pok	TCATATATTTACAGTAGGTATAGATGTAGATACTCGAGCTTATTTTACTT	700
K1_L_174_COI|Aeagypti|West_Pok	TCATATATTTACAGTAGGTATAGATGTAGATACTCGAGCTTATTTTACTT	700
K1_L_150_COI|Aeagypti|West_Pok	TCATATATTTACAGTAGGTATAGACGTAGATACTCGAGCTTATTTTACTT	700
K1_L_107_COI|Aeagypti|West_Pok	TCATATATTTACAGTAGGTATAGATGTAGATACTCGAGCTTATTTTACTT	700
K1_L_103_COI|Aeagypti|West_Pok	TCATATATTTACAGTAGGTATAGATGTAGATACTCGAGCTTATTTTACTT	700
K1_1121_COI|Aeagypti|West_Poko	TCATATATTTACAGTAGGTATAGATGTAGATACTCGAGCTTATTTTACTT	700
K1_1064_COI|Aeagypti|West_Poko	TCATATATTTACAGTAGGTATAGACGTAGATACTCGAGCTTATTTTACTT	700
K1_951_COI|Aeagypti|West_Pokot	TCATATATTTACAGTAGGTATAGATGTAGATACTCGAGCTTATTTTACTT	700

KC690897_Aedes_albopictus_COI	CTGCAACTATAATTATTGCGGTACCT	726
AF390098_Aedes_aegypti	CAGCAACTATAATTATTGCTGTTCCT	726
AY056597_Aeformosus	CAGCAACTATAATTATTGCTGTTCCT	726
Aedes_aegypti_MF194022	CAGCAACTATAATTATTGCTGTTCCT	726
LL_089_COI|Aeagypti|Turkana	CAGCAACTATAATTATTGCTGTTCCT	726
LL_086_COI|Aeagypti|Turkana	CAGCAACTATAATTATTGCTGTTCCT	726
LL_084_COI|Aeagypti|Turkana	CAGCAACTATAATTATTGCTGTTCCT	726
LL_083_COI|Aeagypti|Turkana	CAGCAACTATAATTATTGCTGTTCCT	726
LL_075_COI|Aeagypti|Turkana	CAGCAACTATAATTATTGCTGTTCCT	726
L_04_COI|Aeagypti|Turkana	CAGCAACTATAATTATTGCTGTTCCT	726
KS_L_618_COI|Aeagypti|West_Pok	CAGCAACTATAATTATTGCTGTTCCT	726
KS_L_613_COI|Aeagypti|West_Pok	CAGCAACTATAATTATTGCTGTTCCT	726
KS_L_606_COI|Aeagypti|West_Pok	CAGCAACTATAATTATTGCTGTTCCT	726
KR_L_660_COI|Aeagypti|West_Pok	CAGCAACTATAATTATTGCTGTTCCT	726
KR_L_654_COI|Aeagypti|West_Pok	CAGCAACTATAATTATTGCTGTTCCT	726

K_043_COI|Aeagypti|West_Pokot	CAGCAACTATAATTATTGCTGTTCCT	726
K_040_COI|Aeagypti|West_Pokot	CAGCAACTATAATTATTGCTGTTCCT	726
K_034_COI|Aeagypti|West_Pokot	CAGCAACTATAATTATTGCTGTTCCT	726
K4_L_058_COI|Aeagypti|West_Pok	CAGCAACTATAATTATTGCTGTTCCT	726
K4_L_057_COI|Aeagypti|West_Pok	CAGCAACTATAATTATTGCTGTTCCT	726
K3_L_207_COI|Aeagypti|West_Pok	CAGCAACTATAATTATTGCTGTTCCT	726
K3_L_184_COI|Aeagypti|West_Pok	CAGCAACTATAATTATTGCTGTTCCT	726
K3_L_072_COI|Aeagypti|West_Pok	CAGCAACTATAATTATTGCTGTTCCT	726
K3_1172_COI|Aeagypti|West_Poko	CAGCAACTATAATTATTGCTGTTCCT	726
K3_1168_COI|Aeagypti|West_Poko	CAGCAACTATAATTATTGCTGTTCCT	726
K2_L_093_COI|Aeagypti|West_Pok	CAGCAACTATAATTATTGCTGTTCCT	726
K2_1154_COI|Aeagypti|West_Poko	CAGCAACTATAATTATTGCTGTTCCT	726
K1U_L_060_COI|Aeagypti|West_Po	CAGCAACTATAATTATTGCTGTTCCT	726
K1_L_220_COI|Aeagypti|West_Pok	CAGCAACTATAATTATTGCTGTTCCT	726
K1_L_209_COI|Aeagypti|West_Pok	CAGCAACTATAATTATTGCTGTTCCT	726
K1_L_198_COI|Aeagypti|West_Pok	CAGCAACTATAATTATTGCTGTTCCT	726
K1_L_192_COI|Aeagypti|West_Pok	CAGCAACTATAATTATTGCTGTTCCT	726
K1_L_189_COI|Aeagypti|West_Pok	CAGCAACTATAATTATTGCTGTTCCT	726
K1_L_174_COI|Aeagypti|West_Pok	CAGCAACTATAATTATTGCTGTTCCT	726
K1_L_150_COI|Aeagypti|West_Pok	CAGCAACTATAATTATTGCTGTTCCT	726
K1_L_107_COI|Aeagypti|West_Pok	CAGCAACTATAATTATTGCTGTTCCT	726
K1_L_103_COI|Aeagypti|West_Pok	CAGCAACTATAATTATTGCTGTTCCT	726
K1_1121_COI|Aeagypti|West_Poko	CAGCAACTATAATTATTGCTGTTCCT	726
K1_1064_COI|Aeagypti|West_Poko	CAGCAACTATAATTATTGCTGTTCCT	726
K1_951_COI|Aeagypti|West_Pokot	CAGCAACTATAATTATTGCTGTTCCT	726