Spatial and temporal distribution of *Aedes* mosquitoes, Dengue and Chikungunya Viruses and their Phylogeny along the Coastline of Kenya

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DECLARATION

I declare that this research thesis is my original work and has not been presented for a degree in any other university or any other award.

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DEDICATION

This thesis is dedicated to my beloved parents Mr. Jimmy Ngala Chandugu and Mrs. Mbuche Ngala Mwanyamba.

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ABBREVIATIONS AND ACRONYMS

ARBOVIRUS	Arthropod-Borne Virus
ArcGIS	ArcGeographical Information System
BG TRAP	Bio Gent Sentinel Trap
CHIKV	Chikungunya Virus
CT	Cycle threshold
DENV	Dengue Fever Virus
GPS	Geographical Positioning System
GPSR	Global Positioning System Receiver
MEM	Minimum Essential Media
ML	Maximum Likelihood
mtNAD4	Mitochondrial Nicotinamide Adenine Dehydrogenase sub unit 4 gene
NUMTS	Nuclear Mitochondrial DNA
ORF	Open Reading Frame
qPCR	quantitative/ real time PCR
RAxML	Randomized Axelerated Maximum Likelihood
RT-PCR	Reverse Transcription PCR
WHO	World Health Organization

DEFINITION OF TERMS

Arbovirus: Arthropod-borne viruses which are transmitted to humans primarily through the bites of infected hematophagous arthropods, for example *Aedes* mosquitoes.

Clade: A group of organisms believed to comprise all the evolutionary descendants of a common ancestor.

Cycle threshold: This is the number of amplification cycles needed for the fluorescent signal to cross the threshold level in real time PCR.

Genome: A haploid set of chromosomes in a gamete or microorganism/ or the genetic material of an organism consisting of DNA including both the genes (coding regions) and non-coding DNA as well as the genetic material of the mitochondria.

Genotype: Is a set of genes in a DNA which is responsible for a particular trait or the genetic make-up of an organism.

Haplotype: Is a group of genes within an organism that is inherited together from a single parent. This word is derived from “haploid” which describes cells with only one set of chromosomes.

Monophyletic group: It is a set of all the descendants from a single ancestor, including that particular ancestor.

Orthology: Refers to groups of genes that reveal species phylogeny. Thus, within a monophyletic gene group each species is represented by a single orthologue gene.

Out-group: A group of organisms not belonging to the group whose evolutionary relationships are being investigated. Such a group is used for comparison to assess which characteristics of the group being studied are more widely distributed and may therefore be older in origin.

Paralogues: Set of genes which reveal the history of a gene family. Thus, within a gene group each species may be represented by a number of paralogues.

Paraphyletic group: Monophyletic and paraphyletic groups have a single evolutionary origin. If one lineage emerging from a monophyletic group is removed, a paraphyletic group remains. In contrast, polyphyletic groups result from convergent evolution and the characters that supports the group are absent in the most recent common ancestor.

Phylogenesis: The evolutionary development of an organ or other part of an organism.

Phylogeny: The evolutionary development and history of a species or higher taxonomic grouping of organisms.

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ABSTRACT

There are arthropod-borne disease outbreaks as a result of pathogen influx including arboviruses which are transmitted by strains of *Aedes* species that occur periodically in varying spots in Kenya. However, there has been paucity of documented information on the epidemiology of *Aedes* mosquitoes involved in transmission of different strains of viruses. This cross sectional study determined spatial and temporal distribution of *Aedes* mosquitoes, Dengue and Chikungunya viruses, and their phylogeny and vector-virus co-infections during dry and wet seasons. Indoor and outdoor sampling of adults *Aedes* mosquitoes was done using Biogent Sentinel trap baited with solid carbon dioxide and Prokopack aspiration technique. *Aedes* mosquitoes were identified and sorted according to collection site, sex, physiological status and species using their morphological features and molecular techniques. Sentinel sites coordinates were recorded by Global Positioning System receiver with spatial and temporal maps generated using ArcGeographical information system. RNA was extracted from *Aedes* mosquitoes using Trizol®. Identification of *Aedes* species, Dengue and Chikungunya was done using Polymerase Chain Reaction. Sequencing of amplicons was done using Sanger high-throughput technique and their proportions analysed by R-statistics. Phylogeny tree files were generated using Randomised Accelerated Maximum Likelihood and trees plotted using interactive tree of life. A total of 37,220 *Aedes* mosquitoes belonging to eight species were collected and grouped in pools of 20 mosquitoes. *Aedes aegypti formosus* was dominant at 62.5%. *Aedes aegypti aegypti* was identified for the first time along the Coastline of Kenya. There was no effect of season on the distribution and proportion of *Aedes* species along the Coastline. *Aedes* mosquitoes belonged to the upper clade of the phylogenetic tree. Four serotypes of Dengue virus were identified with DENV-4 identified for the first time in *Aedes* mosquitoes in the region. Only the East/Central/South African (ECSA) genotype of Chikungunya virus was isolated and seasons did not influence the distribution of both viruses along the Coastline ($p>0.001$). *Aedes* mosquitoes were closely related to previous isolates and to those from Uganda, Senegal and Thailand. DENV-1 isolates were closely related to those from India, DENV-2 isolates were closely related to those from Pakistan, and DENV-3 isolates were closely related to those from Brazil while DENV-4 isolates were closely related to those from Haiti. Chikungunya ECSA genotype isolates were closely related to previous Kenyan isolates and to those from South Africa and Tanzania. There were co-infections of Dengue and Chikungunya viruses in *Aedes* mosquitoes. *Aedes aegypti s.l* and *Aedes pembaensis* had co-infections of all viruses. Prevalence of Dengue virus was at 7.9% while Chikungunya was at 2.1%. These results are important as they give information on areas of high risk for the virus outbreaks. Surveillance of entomological infection by viruses and implementation of their appropriate control measures should be taken by the Ministry of Health.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Mosquitoes are responsible for transmission of many pathogens including viruses, bacteria and parasites between people, domestic and wild animals all over the world (Kraemer *et al.*, 2015). Viruses transmitted by arthropods are commonly known as arboviruses, and they are primarily transmitted to humans and animals through bites of infected hematophagous arthropods, for example, mosquitoes in the genus *Aedes* (Lindsey *et al.*, 2014). This group of viruses is maintained in the zoonotic cycle with human beings acting as incidental dead-end hosts (Bravo *et al*, 2014). This implies humans have a limited role in the maintenance of the virus life cycle (Ochieng *et al.*, 2013).

In the ecosystem, the virus life cycles are maintained through intrinsic and extrinsic incubation phases (Appendix 1). Example of such viruses are: Dengue fever virus, Chikungunya virus, Rift valley fever virus, West Nile virus, Zika virus, Yellow fever virus, Japanese encephalitis virus and Venezuelan equine encephalitis virus among others (Gubler, 2002; Sang *et al.*, 2017). These viruses cause a ranging degree of clinical syndromes with different severity in humans and domestic/wild animals (Ranjit & Kissoon, 2011). Infections range from being self-limiting characterized by fevers to deadly histopathological manifestation like encephalitis and/or haemorrhagic fevers (Rico-Hesse, 2009).

1.1.1 Life cycle and bionomics of Mosquitoes

Mosquitoes have a complete life cycle with four main stages: egg, larvae, pupa and adult (Day, 2016). The egg, larvae and pupa are aquatic stages while the adults are terrestrial (Appendix 2). The larvae stage exhibit four larval instars and are the aquatic feeding stage of mosquitoes. Before the female mosquito oviposit its eggs, a blood meal is crucial for viability of its eggs. Feeding behaviours of the female mosquitoes differ among genera (Harrington *et al.,* 2014). Some mosquitoes obtain their blood meals during the night thus referred to as nocturnal, for example, mosquitoes in the genus *Anopheles* which are mainly involved in transmission of malaria parasite. Others obtain their blood meal during the day thus referred to as diurnal, for example, mosquitoes in the genus *Aedes*. Some prefer obtaining blood meals from human being thus referred to as anthropophagic, some from animals thus referred to as zoophilic.

Mosquitoes in the genus *Aedes* are diurnal, exophagic and exophilic (prefer feeding and resting outdoors) with preference to feeding early in the morning and late in the afternoon on both humans and livestock (Chadee, 2012). After a blood meal, the female mosquitoes take shelter under bushes of shrubs or trees to digest their meal and become gravid. They then oviposit their eggs on aquatic environment preferably in black containers thus the name “container mosquitoes” (Agha *et al.,* 2017). Selection of the oviposition site is crucial to ensure survival of the eggs and larvae as most aquatic ecologies are associated with dangers of drying up and scavengers (Day, 2016). The eggs float and hatch into larvae which develop into pupae and into adults with the entire life cycle taking an average of 10 to 14 days depending on temperature and humidity.

1.1.2 Taxonomy of *Aedes* mosquitoes

Aedes mosquitoes belong to Phylum Arthropoda, class Insecta, order Diptera, family Culicidae and genus *Aedes* Meigen, (Meigen, 1818). A sister genus to *Aedes* referred to as *Ochlerotatus* Lynch Arribalzaga, (Lynch Arribalzaga, 1891) was proposed based on morphological differences at the 4th stage of the larvae and pupae. Previously, taxonomy of living organisms was based on visible morphological traits but molecular tools are currently used for identification, classification and studying of evolutionary relationships among organisms.

Taxonomy of *Aedes* mosquitoes has been based on similarity and differences in their morphological orientations (Edward, 1941; Mattingly, 1967). The utilization of morphological features has contributed immensely in analysis and identification of *Aedes* mosquitoes at genus and species level (Shepard e*t al.,* 2006). However, new effective tools such as use of allozymes and molecular genetic markers have contributed immensely in identification and plotting of phylogenetic trees of organisms, including *Aedes* mosquitoes. Such molecular genetic markers include microsatellites and mitochondrial markers which provides a more detailed and reliable information about their identity and phylogeny (Minard *et al.,* 2017).

1.1.3 Epidemiology of *Aedes* mosquitoes

There are two species of *Aedes* mosquitoes which have been identified to be primary vectors of Dengue and Chikungunya viruses all over the world (Kraemer *et al.,* 2015). These species are: *Aedes aegypti* s.l and *Aedes albopictus* also known as the “Asian tiger” mosquito. *Aedes aegypti* s.l mainly transmits arboviruses in sub Saharan, tropical and sub-tropical regions while *Aedes albopictus* is mainly involved in the transmission of the viruses in the Asian continent (Coelho, 2015). *Aedes aegypti* s.l consists of two sub species; *Aedes aegypti formosus* and *Aedes aegypti aegypti* (Moore *et al.,* 2013). *Aedes aegypti formosus* is the ancestral *Aedes* species and mainly inhabits forested areas feeding on blood from animals like monkeys (a sylvan mosquito). It is geographically distributed within the sub Saharan Africa. However, *Aedes aegypti aegypti* is geographical distributed globally in the tropical and sub-tropical regions and is mainly associated with humans (Mattingly, 1967).

1.1.4 Mechanisms of *Aedes* mosquitoes in transmission of viruses

Aedes species have evolved to be effective in maintenance of viruses in human and vector population due to their ability to incubate the viruses (Bravo *et al.,* 2014). *Aedes* mosquitoes have capacity to transmit viruses through three main mechanisms: i) vector-host-vector interaction ii) sexual mating following emergency from pupae and iii) transovarial (da Costa *et al.,* 2017). *Aedes* mosquitoes acquire the infection following a blood meal from an infected human or animal and the virus replicates in the tissues of the mosquito to reach the salivary glands. The virus is subsequently transmitted to the next host through a bite when the infected mosquito takes a blood meal (Harrington *et al.,* 2014). The virus can also be transmitted within mosquito populations through transovarial route as well as during mating of the young ones 2-3 days post emergence from the pupae stage (da Costa *et al.,* 2017). Therefore, the males only transmit the viruses to their female counterparts during mating following emergence from the pupa stage.

1.1.5 Epidemiology of Arboviruses

Epidemiology of arboviruses is partly dependent on the ecology and presence of vector species responsible for their transmission (Njenga *et al.,* 2008). Therefore, to effectively determine the distribution of arboviruses in relation to time and space, screening of potential mosquito vectors is paramount as they act as good indicators of areas at risk for arboviral disease outbreak (Grech, *et al.,* 2010). This is because the viruses exist in the tissues of these mosquitoes during their entire life span. In addition, outbreaks and/or disease incidences occur in human and animal populations when there is favourable environmental condition for the development of the vectors (Coelho, *et al.,* 2015). For example, prolonged rainfalls provide abundant breeding sites and increased socio-economic activities including cultivating, working and grazing in bushes where *Aedes* mosquitoes rest during the day thus increasing the risk of vector-human contacts and virus infection (Agha *et al.,* 2017).

Prompt isolation and identification of arboviruses within the population of mosquito vectors is paramount for effective management of arboviral infections in human populations, as this forms a basis for early warning systems allowing appropriate measures to be put in place to prevent outbreaks of arboviral infections (Coelho *et al.,* 2015). Thus, detection can be achieved through surveillance programs tailored towards monitoring of virus activity in the vector candidates (Grech *et al.,* 2010). Such surveillance provides a platform for mapping of the disease distribution as well as knowledge necessary for assessing risk and identification of mosquito species responsible for transmission of the viruses (Ochieng *et al.,* 2013).

1.1.6 Biology of Dengue virus

Dengue virus was first reported between the years 1779 and 1780 in the Asian, African and the America continents (Gubler and Clark, 1995). This virus belongs to the family of *Flaviviridae* and genus *Flavivirus*. Dengue virus consists of an envelope measuring 40-60 nm in size with an isometric nucleocapsid measuring 25-30 nm with approximately 10.7 kb linear, positive-sense RNA genome (Paranjape and Harris, 2010). Currently, there are four distinct serotypes of Dengue virus ratified by the International Committee on Taxonomy of Viruses (ICTV): DENV-1, DENV-2, DENV-3 and DENV-4 (Normile, 2013). These serotypes can be differentiated based on their surface antigens resulting in production of different antibodies by the human immune system.

There are different genotypes within each Dengue serotypes exhibiting greater genetic variance. For example, the "Asian" genotypes including DENV-2 and DENV-3 are major causes of severe Dengue fever infections especially when an individual is experiencing a secondary infection of Dengue virus (Kosasih *et al.,* 2016). The virus genome contains about 11, 000 nucleotides coding for three proteins which are part of the structural proteins including C, prM and E, involved in assembly of the virus particle (Guzman *et al.*, 2010). In addition, there are non-structural proteins in host viral infected cells including; NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5 which are crucial for virus replication (Rodenhuis-Zybert *et al.,* 2010).

1.1.7 Pathogenicity of Dengue virus

All serotypes of Dengue virus can cause severe disease, that is, Dengue fever (DF), Dengue haemorrhagic fever (DHF) and Dengue shock syndrome (DSS) (Rico-Hesse, 2009). Infection by one of the Dengue serotypes does not confer immunity against the others; therefore co-infections can occur (Gubler and Clark, 1995). DHF and DSS are defined by four clinical manifestations; high fever, haemorrhagic diathesis, hepatomegaly and shock (Kraus *et al.,* 2003). Dengue infection is categorised in relation to severity of the infection (Martina *et al.,* 2009), that is, relatively mild cases without shock, with grades I and II fever are referred to as DHF; in severe cases with shock, grade III and IV are referred to as DSS. If the patients are not well treated and managed, grade IV dengue virus can cause severe shock where the patient pulse and blood pressure become undetectable and death can occur within 12 to 36 hours (Yung *et al.,* 2015). The mortality rate for DHF is estimated to be 20% (Rico-Hesse, 2009). However, DENV-1 primary infection may be more severe compared with DENV-2 (Yung *et al.,* 2015).

1.1.8 Epidemiology of Dengue virus

Dengue virus infects a wide human population world over of up to 528 million people a year resulting to approximately 25,000 deaths (Bhatt *et al.*, 2013). However, Dengue virus disease is still considered as a neglected tropical disease by the World Health Organization (Murray *et al.,* 2013). The virus can adopt two different life cycles of transmission and amplification, that is, sylvan and urban (Martina *et al.,* 2009). In the sylvan cycle, the virus undergoes rounds of infection, amplification and re-infection between non-human primates and arthropod vectors. The infected arthropod vectors then migrate to the human settlements and initiate the urban cycle in which the cycles of infection, amplification and re-infection occur between the human and vector population.

In urban set ups like cities and towns, Dengue virus is mainly transmitted by the *Aedes aegypti* which is more of a domesticated *Aedes* mosquito. Within the rural areas, Dengue virus is mainly transmitted to humans by *Aedes aegypti* as well as other species such as *Aedes albopictus* (Gubler, 2002). The assessment of health, social and economic impact of Dengue virus infections is difficult to determine due to impediments such as challenges in disease surveillance, inaccurate diagnosis and poor reporting of outbreaks (Murray *et al.,* 2013).

Dengue virus together with its associated arthropod mosquito vectors has a wide geographical range (Grard *et al.,* 2014). The virus is mainly present in the tropical and subtropical zones of the African and Asian continents with endemicity in more than 125 countries (Murray *et al.,* 2013). This expansive geographical distribution is associated with the high incidence of epidemics and hyperendemicity and it is influenced by parameters like changes in weather and climatic conditions, globalization, travel, trade, socioeconomics, settlement and mutations within the virus population (Kosasih *et al.,* 2016). And as the virus increase in its geographical niche, there is a possibility of evolution to more severe forms (Kraemer *et al.,* 2015).

1.1.9 Biology of Chikungunya virus

The etymology of Chikungunya virus is from the Makonde people of Tanzania translated to mean "that which bends up" due to the stooped posture associated with pain of the disease (Kajeguka *et al.*, 2016). Chikungunya is one of the arthropod-borne virus belonging to the family of *Togaviridae* and genus *Alphavirus* (Thiberville, *et al.,* 2013a). The virus has three (3) genotypes having been characterised: West African (WAG), East/Central/South African (ECSA) and Asian genotypes (AG) (Lanciotti & Valadere, 2014). In Kenya, only the ECSA genotype has been reported (Njenga *et al.,* 2008).

Chikungunya virus is a positive-sense single stranded RNA virus approximately 11.8 kb long (Rougeron *et al.,* 2015). It is comprised of an icosahedral capsid which is covered by a lipid layer of diameter 65 nm and very sensitive to temperatures of above 58°C (Thiberville *et al.*, 2013a). The virus has two open reading frames (ORFs), that is, one on the 5’ end and the other on the 3’ end (Kumar *et al.*, 2015). The ORF located at the 5’ end produces four non-structural proteins (NSP 1-4) and the ORF on the 3’ end produces the structural proteins which consists of a capsid protein, two envelope glycoproteins (E1 and E2) and two small cleavage products (E3 and 6K) (Petitdemange *et al.,* 2015).

1.1.10 Pathogenicity of Chikungunya virus

Like other arboviruses, Chikungunya infection may result into a fever-rash-arthralgia syndrome in humans (Petersen *et al.,* 2016). This arthralgia has the potential to persist for some few months or even years depending on the immune status of the affected person (Thiberville *et al.,* 2013b). In some cases, the infection may progress to arthritis where there is inflammation in addition to pain. About 72-97% of the infected individuals infected by Chikungunya become symptomatic (Sharp *et al.,* 2014). Only about 18% of the infected persons can manifest clinical illness associated with re-exposure to the virus (Yoon *et al.,* 2015). Though the disease is characterised by low mortality rate, the symptoms and chronic nature is a clear indication that the disease should not be ignored (Ganesan *et al.,* 2017).

1.1.11 Epidemiology of Chikungunya virus

Chikungunya is present in many countries of Africa, Asia and the Indian sub-continent and parts of Europe (Lanciotti and Valadere, 2014). The higher rate of geographical expansion is facilitated by increase in global travels and migration surge (Ganesan *et al.,* 2017). The primary vectors for Chikungunya are *Aedes* mosquitoes; *Aedes Aegypti* in Africa and *Aedes albopictus* in Asian (Coelho, 2015). Globally, Chikungunya causes an estimate of over 1 million morbidity cases per year with a fatality rate of about 0.1% (Petersen *et al.,* 2016).

Chikungunya fever re-emerged in the form of devastating epidemics in and around the Indian Ocean in 2005. These outbreaks were linked to mutations in the viral genome which aided replication of the virus in *Aedes albopictus* mosquitoes (Thiberville, *et al.,* 2013b).

In Africa, Chikungunya is spread via a zoonotic cycle or Dengue-like cycle (Zeller *et al.*, 2016). Zoonotic cycle involves transmission of the virus from a non-human primate reservoir and sylvatic mosquitoes with humans being incidental or dead end hosts. However, the Dengue-like cycle involves direct human-mosquito-human transmission by peridomestic *Aedes* species. This means the virus largely resides in other primates in between human outbreaks (Lindsey *et al.,* 2014).

1.1.12 Phylogeny

Phylogeny refers to analysis and description of an evolutionary history of a group of organisms (Harrison & Langdale, 2006). In biological studies, the primary goal of developing an evolutionary tree is to describe the evolutionary relationships between or among organisms based on relative recency of common ancestry (Kitching *et al.,* 1998). These evolutionary relationships are depicted in a cladogram tree with branches joined by nodes which lead to terminals at the ends of the tree branches. Organisms on the phylogenetic tree exhibit three major forms of evolutionary relationships between or among themselves; monophyly, paraphyly and polyphyly (Hennig, 1966; Fitch, 1970).

Phylogenetic analysis using analytical tools like parsimony phylogenetics could provide information on the common genetic changes (mutations) and modifications in biochemical pathways (Abu-Asab *et al.*, 2008). Such information could be crucial in connecting omics and study of arboviral diseases by providing seamless, predictive, dynamic and multidimensional analysis which can be utilised for timely diagnosis, detection, and prognosis, identification of biomarkers and determination of effective treatment.

1.1.13 Co-infection of viruses in *Aedes* mosquitoes

Arthropod virus co-infection refers to presence of more than one virus in a vector such as mosquito which has the potential to affect the pattern of pathogen transmission and virulence (Le Coupanec *et al.*, 2017). More so, co-infection can be regulated within the vector hosts by interactions such as cross-protective immunity or at a broader spatial scale via vector distributions (Seabloom *et al.*, 2009). Ecological parameters conducive for breeding of *Aedes* mosquitoes and development of viruses within their midgut have immensely contributed to invasion and circulation of various viruses in the same geographical niche (Chaves, *et al.*, 2018). People living in these areas endemic for arboviruses have been simultaneously infected with more than one virus (Ruckert *et al.*, 2017).

Zika virus, Dengue and Chikungunya viruses can exist in the same geographical regions and vectored by the same *Aedes* mosquito (Göertz, *et al.*, 2017). Similarly, *Aedes aegypti* can transmit both Zika and Chikungunya viruses via a single bite to humans without any influence on their vector competence (Göertz, *et al.*, 2017).

1.2 Statement of the Problem

Studies on distribution, composition, proportions and phylogeny of *Aedes* mosquitoes and entomological detection of Dengue and Chikungunya viruses on the entire Coastline of Kenya had not been done. Similarly, identification of serotypes of Dengue and genotypes of Chikungunya viruses and their evolutionary relationship had not been done. The few earlier studies on Dengue and Chikungunya were based on serological assays (IgM and IgG) of patients' blood presenting with fever at various health facilities. Furthermore, earlier studies sampled limited geographical areas (Msambweni, Rabai, Kilifi, Malindi and Lamu) within the coastal region for short durations of time. In addition, information of concomitant infections of Dengue and Chikungunya in different *Aedes* species was not available, and thus knowledge on how each species influences epidemiology the viruses was unknown.

1.3 Justification of the study

There has been periodic outbreaks of arboviral diseases all over the world (Kosasih *et al.*, 2016). In Kenya, an outbreak of Dengue fever was first reported in 1982 in Malindi and Mombasa (Johnson *et al.,* 1982). Previously, Dengue outbreaks occurred in 2011 in Northern Kenya and later in 2013-2014 in Mombasa (Konongoi *et al.,* 2016). Recently, outbreaks of Dengue fever occurred in April 2017 and May 2018 (www.health.go.ke). An outbreak of Chikungunya fever was reported in Lamu Island of Kenya for the first time in 2004 (Moore *et al.,* 2013). Recently, Chikungunya fever outbreak was reported in Mombasa County in May 2018 (www.health.go.ke). *Aedes aegypti* was identified as the primary vector in the transmission of these viruses (Moore *et al.,* 2013; Konongoi *et al.,* 2016). In view of these outbreaks, information on composition, abundance and distribution of *Aedes* species in relation to space and time and their arboviral loads is crucial in designing of effective management strategies for their control. In this regard, stakeholders such as the Ministry of Health and Medical Research Institutions can strengthen surveillance and screening for arboviruses and their vectors in areas with high prevalence. Knowledge on phylogeny is essential to estimate how pathogen vectors are related in order to determine common or different control and management strategies. In addition, assessment of co-infections by various serotypes of Dengue and genotypes of Chikungunya in *Aedes* species provides insight into the epidemiology of the disease. This knowledge ensures efforts and resources for management of Dengue and Chikungunya infections by the Ministry of Health and relevant stakeholders are utilised economically and appropriately for improved human health.

1.4 Research Questions

i. What is the distribution of *Aedes* mosquitoes in relation to space and time along the Coastline of Kenya?
ii. What is the phylogeny of *Aedes* mosquitoes found along the Coastline of Kenya?
iii. What is the phylogeny of Dengue and Chikungunya viruses found along the Coastline of Kenya?
iv. Are there co-infections of Dengue and Chikungunya viruses in *Aedes* mosquitoes found along the Coastline of Kenya?

1.5 Objectives

1.5.1 General Objective

To identify and determine spatial and temporal distribution of *Aedes* species, Dengue and Chikungunya viruses and their phylogeny along the Coastline of Kenya.

1.5.2 Specific Objectives

i. To identify and determine spatial and temporal distribution of *Aedes* mosquitoes along the Coastline of Kenya.

ii. To understand phylogeny of *Aedes* mosquitoes found along the Coastline of Kenya.

iii. To understand phylogeny of Dengue and Chikungunya viruses in *Aedes* mosquitoes found along the Coastline of Kenya.

iv. To determine co-infections of Dengue and Chikungunya viruses in *Aedes* mosquitoes found along the Coastline of Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 Mosquitoes for transmission of Arboviruses

Arboviruses solely depend on their vectors for transmission from one vertebrate host to the other (Petersen *et al.*, 2016). In this case, Dengue and Chikungunya viruses depend on *Aedes* mosquitoes for their transmission among individual human beings (Rückert *et al.*, 2017). Transmission of the viruses occur during a blood meal by the female mosquitoes from one vertebrate host to the other as the virus passes through the proboscis from the salivary glands to the host (Harrington *et al.*, 2014). Therefore, surveillance activities to determine presence of *Aedes* mosquitoes in a given ecosystem are crucial to estimate the potential of arboviral transmission to the human population (Ochieng *et al.*, 2013). Globally, *Aedes* species have been reported to be responsible for outbreaks of arboviral infections, with *Aedes aegypti* and *Aedes albopictus* as primary vectors (Weeratunga *et al.*, 2017).

2.2 Distribution of *Aedes* mosquitoes

The global distribution of various *Aedes* mosquitoes is shown in Figure 2.1: *Aedes aegypti* and *Aedes albopictus* are distributed in four continents of America, Africa, Asia and Australia. In America, the vectors are present in Central and latin America. In Africa, the vectors are found within the tropical region. In Australia, the vectors are concentrated in northern and eastern districts, while in Asia, the vectors are mainly found in south eastern region.

Aedes aegypti has been reported to be responsible for transmission of Zika virus in the United States of America (Harrington *et al.*, 2014; da Costa *et al.*, 2017). In Africa, *Aedes aegypti* has been reported to be the primary vector for arboviruses (Paupy *et al.*, 2012). The major *Aedes* mosquitoes in west Africa are *Aedes aegypti aegypti* while *Aedes aegypti formosus* are mainly found in East Africa (Moore *et al.*, 2013). In Kenya, this mosquito vector alongside other *Aedes* species have been reported to be responsible for transmission of viruses: *Aedes albicosta*, *Aede fryeri and Aedes fulgens*; (Edwards, 1941); *Aedes aegypti aegypti* (Trpis and Hausermann, 1986); *Aedes pembaensis* (Sang *et al.*, 2010); *Aedes mcnitosh*, *Aedes ochraceous* and *Aedes tricholabis* (Ochieng *et al.*, 2013)*; Aedes aegypti formosus* (Moore *et al.*, 2013).

Aedes albopictus has been reported to be the primary vector for viruses including Dengue and Chikungunya in the Asian continent (Vazeille *et al.*, 2010). In support of the reports, this mosquito vector was previously reported in La Reunion Island (Minard *et al.*, 2017). Currently, *Aedes albopictus* has expanded its geographical distribution and has been reported in four continents as shown in Figure 2.1 (Kraemer *et al.*, 2015). In Africa, the presence of *Aedes albopictus* was recently reported in Gabon and was responsible for transmission of Zika virus (Grard *et al.*, 2014). The presence of this *Aedes* mosquito has not been reported in Kenya.

It is important to note that an *Aedes* mosquito becomes infectious once infected with the respective viruses: Dengue, Chikungunya or Zika. However, information on spatial and temporal distribution of *Aedes* species along the Coastline of Kenya had not been published. Therefore, there was no data for reference on the distribution of the *Aedes* mosquitoes along the Coastline of Kenya. This was despite reports of positive serological studies for arboviral infections in humans (Njenga *et al.,* 2008; Sutherland *et al.,* 2011; Moore *et al.,* 2013; La Beaud *et al.,* 2015; Ellis *et al.,* 2015; Ngoi *et al.,* 2016; Konongoi *et al.,* 2016; Lutomiah *et al.,* 2016). This means, there was need to survey the composition, abundance and distribution of *Aedes* mosquitoes and their infection status along the Coastline to identify areas that could be at risk of arboviral infections.

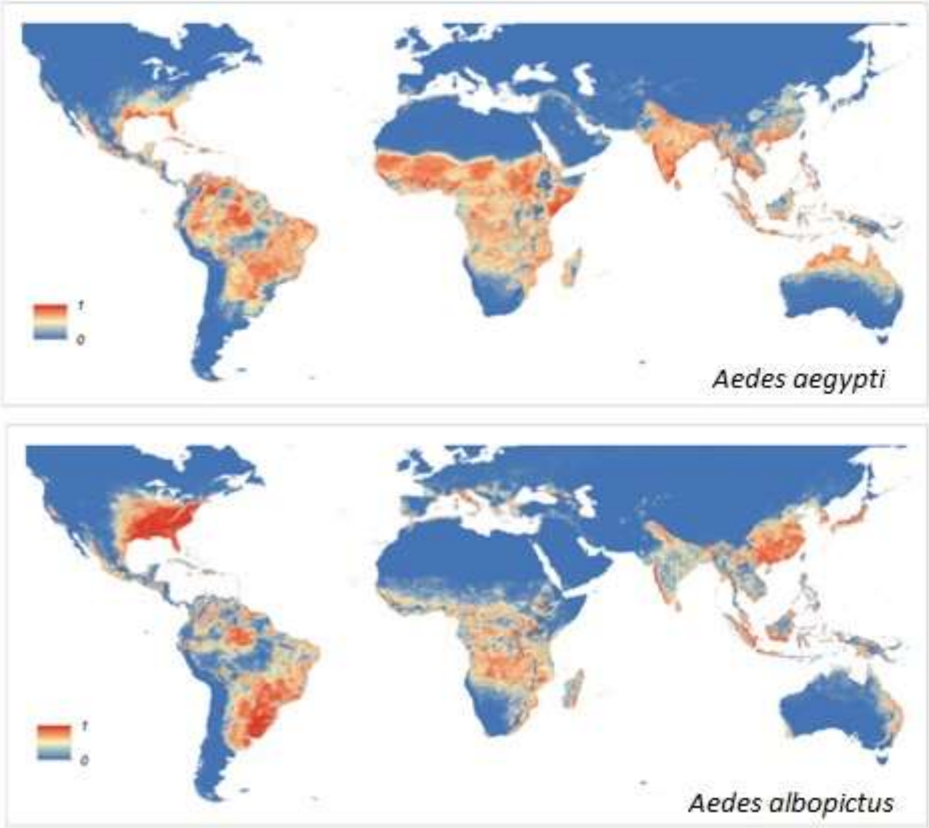


Figure 2.1: Global distribution of primary *Aedes* mosquitoes {Adapted from (Kraemer *et al.*, 2015)}. The map depicts the probability of occurrence (from 0 blue to 1 red) at a spatial resolution of 5 km × 5 km.

2.3 Phylogeny of *Aedes* mosquitoes

The mitochondrial Nicotinamide Adenine dinucleotide dehydrogenase sub unit 4 (ND4) gene has been widely used for construction of phylogenetic trees in various mosquito studies (Moore *et al.,* 2013; Ali *et al.,* 2016). An increase in gene flow among *Aedes aegypti* populations between Africa and Saudi Arabia was previously reported; with phylogenetic relationships indicating that the two genetically distinct *Aedes aegypti* present in Saudi Arabia acquired their origin from the dual African ancestors (Ali *et al.*, 2016). In Africa, phylogeny of West and East African isolates of *Aedes aegypti* s.l indicated existence of two clades of origin: the basal and derived/ upper clade: the basal clade had isolates mainly from West Africa while the upper clade had isolates mainly from East Africa (Figure 2.2). However, one out of the seven isolates from Kenya was in the basal clade while one out of sixteen isolates from Senegal fell in the upper clade. Similarly, the isolate from Uganda was in the lower clade.

These findings from their study clearly demonstrated *Aedes aegypti* mosquitoes all over the world were composed of mosquitoes originating from one of the two matrilineages (basal and derived) and that there existed an evolutionary relationship among *Aedes aegypti* s.l populations from west and East Africa. In their study, a total of 95 unique mtDNA haplotypes were reported, with 24 new haplotypes discovered (16 in Senegal, 1 in Uganda and 7 in Kenya) (Figure 2.2). However, how each of the identified haplotypes influences transmission and epidemiology of the viruses was not analysed.

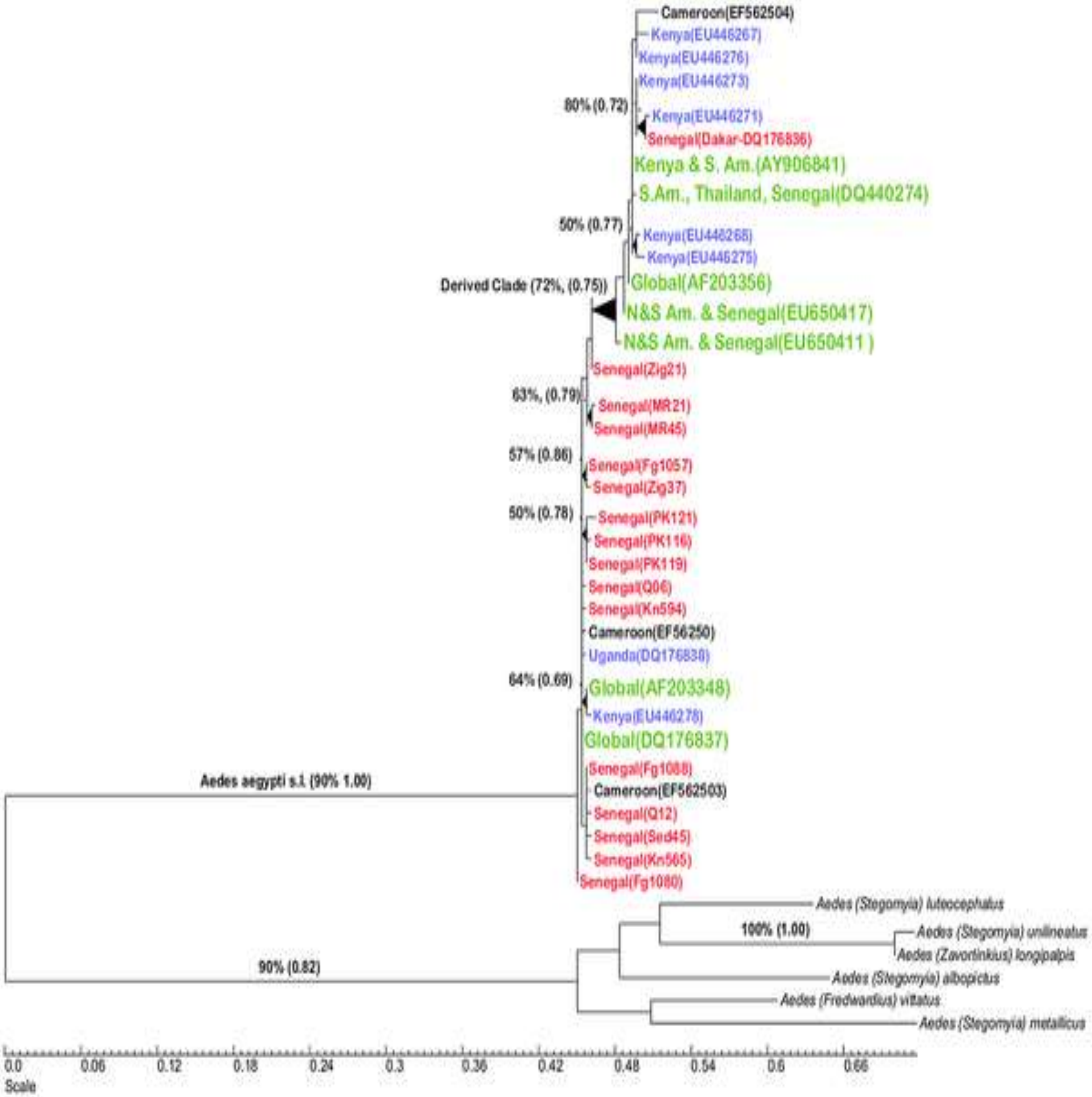


Figure 2.2. Maximum likelihood tree based on the 34 mtDNA ND4 of *Aedes aegypti* present in Africa and out groups {Adapted from (Moore *et al.*, 2013)}.

2.4 Serotypes of Dengue virus and their Global distribution

Dengue virus was first isolated between the years 1779 and 1780 in Asian, Africa and America (Gubler & Clark, 1995). Serotypes of this virus were characterised and currently there are four known serotypes: DENV-1, DENV-2, DENV-3 and

DENV-4. All these serotypes are infectious to human and were isolated from patients presenting with fever at various health facilities all over the world (Sutherland *et al.,* 2011; Ellis *et al.,* 2015; Ngoi *et al.,* 2016; Konongoi *et al.,* 2016). Recently, a new serotype (DENV-5) was isolated in non-human primates (macaque monkeys) in Malasyia although human infections were not reported (Normile, 2013) thus not yet ratified as a human pathogen by the International Committee on Taxonomy of Viruses (ICTV). These serotypes produce different human-immune reactions due to different surface antigens (Yung *et al.,* 2015).

Dengue has been found in four continents of the globe (America, Asia, Africa and Australia) as shown in Figure 2.3: America, Asia and Australia have all four serotypes while Africa has only DENV-1 and DENV-2 serotypes (Murray *et al.,* 2013). This is in contradiction with recent reports by Konongoi *et al.,* (2016) that Kenya has DENV-1, DENV-2 and DENV-3. DENV-3 and DENV-2 were reported to be introduced from the Americans to the Pacific Islands in the years 1964 and 1971 respectively. The pacific island and Philippines were reported to have all four serotypes of Dengue due to their slow introduction from Asia (Bravo *et al.,* 2014). In Saudi Arabia, DENV-2 was identified and reported in the year 1994 (Gubler & Clark, 1995). In Indonesia, all four serotypes of Dengue were reported among people working in large factories in West Java (Kosasih *et al.,* 2016). Since 1780, Dengue has been reported in various African countries: in Mozambique in 1985, in Djibouti in 1991-92, in Somalia in 1982 and 1993 and in Kenya in the year 1982 (Johnson *et al.*, 1982).

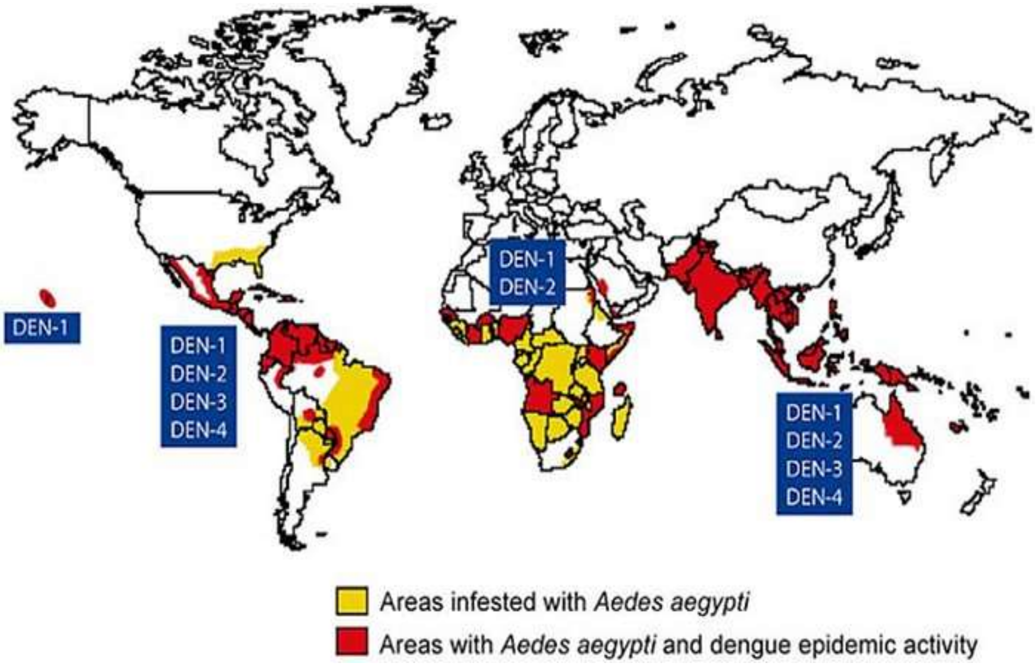


Figure 2.3: Global distribution of serotypes of Dengue virus {Adapted from (Murray *et al.,* 2013)}.

2.5 Serotypes of Dengue virus and their distribution in Kenya

Arboviruses such as Dengue, Chikungunya, Rift valley, West Nile, Usutu, Chaonyang, Yellow fever and Quang Binh have been isolated from serological specimens in Kenya (Ochieng *et al.,* 2013; Sang *et al.,* 2017). Serotypes and genotypes of Dengue virus have been reported from serological studies by Konongoi *et al.,* (2016) as shown in Figure 2.4: Individuals seropositive for DENV-1 were reported in Nairobi, Mombasa, Malindi, Lamu, Wajir and Mandera. DENV-1 and DENV-3 were reported in Nairobi, Mombasa and Mandera while DENV-2 was isolated in Nairobi, Mombasa, Lamu, Garissa, Wajir and Mandera.

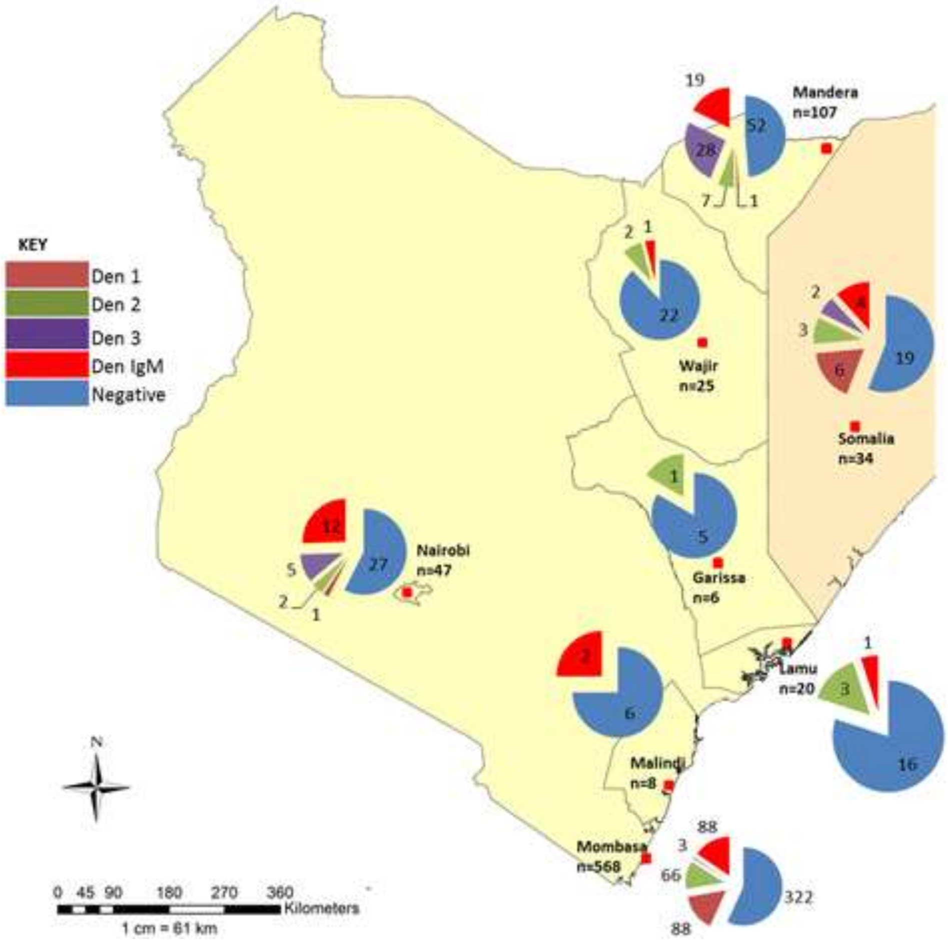


Figure 2.4: Distribution of serotypes of Dengue virus in Kenya {Adapted from (Konongoi *et al.*, 2016)}.

2.6 Epidemic of Dengue virus

In the last two decades, there have been numerous reports on outbreaks of arboviral infections that affect both human beings and their domestic animals all over the world (Gubler, 2002). Dengue fever was reported to be the major cause of mortality in children in Asia and South America (Ranjit and Kissoon, 2011). More so, the viral infection has been reported in over 100 countries. Dengue fever due to infections by DENV-1, DENV-2, DENV-3 and DENV-4 serotypes has increased in Africa since 1980 (Murray *et al.,* 2013). This increase has been associated with low scale surveillance for Dengue infections in the African continent. Recently, there has been up scaled Dengue viral activity within the East Africa region with increased reports on major epidemics outbreaks of Dengue virus infection (Lutomiah *et al.,* 2016).

Major epidemics of Dengue fever were reported for the first time in Seychelles in 1977 and Kenya in 1982 (Johnson *et al.,* 1982). In the year 2011, Kenya experienced a Dengue fever outbreak in the northern region of Mandera and later in coastal region of Mombasa between the years 2013 and 2014. This virus was reported to have entered Kenya through Somalia (Konongoi *et al.,* 2018). Following the 2013-2014 Dengue fever outbreak at the coastal Kenya, an epidemiological map was generated (Lutomiah *et al.,* 2016) (Figure 2.5): the virus was reported in south coast, Mombasa island and north coastal regions. Since then, more outbreaks have been reported in almost every year in the coastal town of Kenya (www.health.go.ke).

Dengue virus has been reported to have a seroprevalence of 12.5% in Kenya (Ochieng *et al.,* 2015). Seroprevalence of the virus in the coastal region was reported to be at 8.8% (Ngoi *et al.,* 2016). However, there were two conflicting reports on seroprevalence of the serotypes of Dengue: DENV-2 was reported to have the highest seroprevalence (Sutherland *et al.,* 2011); while in another report, DENV-1 was reported to have the highest seroprevalence (Konongoi *et al.,* 2016).

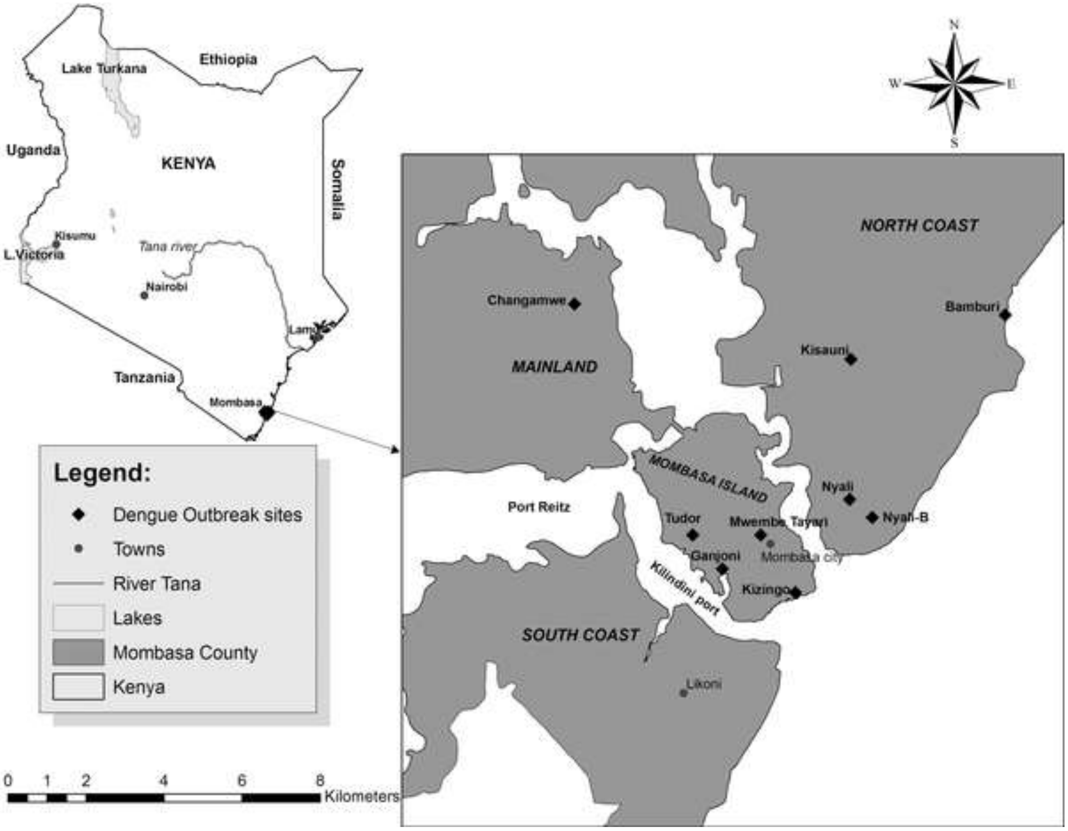


Figure 2.5: Areas with recent Dengue virus outbreaks in Kenya {Adapted from (Lutomiah *et al.,* 2016)}.

2.7 Phylogeny of Dengue virus

Existence of phylogenetic relationships were demonstrated among vector-borne flaviviruses in Kenya (Ochieng *et al.,* 2013). The identified isolates (highlighted in yellow) were subjected to comparison with those in the global database (GenBank) as shown in Figure 2.6: Isolates from Kisumu, Rabai and Garissa were all related among themselves and to the global isolate (gb/jQ068102.1/Chaonyang virus) and gb/GQ165810.1/CFA). Flavivirus isolates from Kisumu were closely related to those from Rabai. West Nile virus isolates from Garissa clustered together thus a close evolutionary relationship. Dengue virus formed a monophyletic cluster with Yellow fever virus displaying close phylogeny between them.

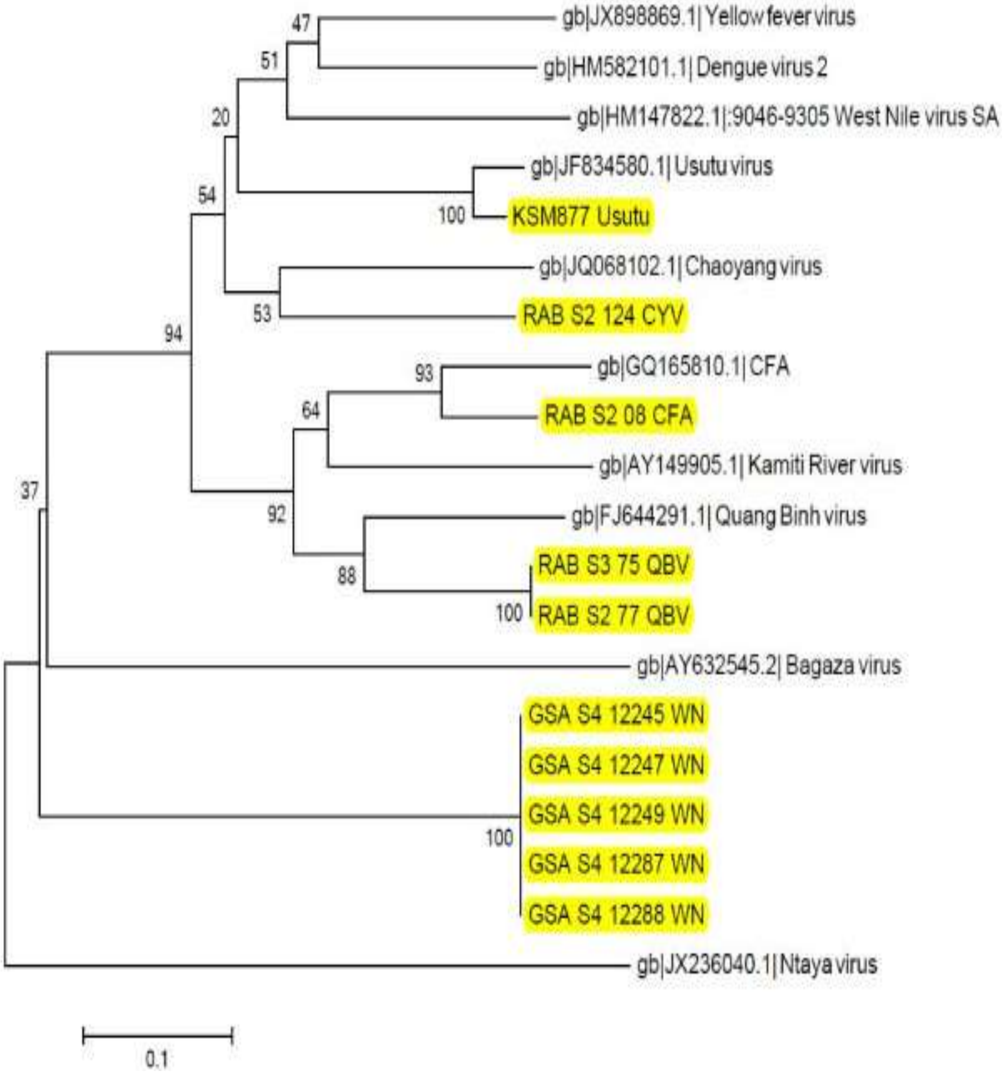


Figure 2.6: Phylogeny of isolates of Flaviviruses {Adapted from (Ochieng *et al.*, 2013)}.

Phylogenetic analysis of three serotypes of Dengue virus (DENV-1, DENV-2 and DENV-3) from Northern and Coastal regions of Kenya was done (Konongoi *et al.*, 2016) as shown in Figure 2.7: DENV-1 isolates from Mombasa were related to those from Djibouti, China and Indonesia. DENV-2 isolates from Mombasa originated from those of West Africa: Senegal and Burkina Faso, and were related to those from Asia: Pakistan, India, Indonesia, China and Singapore. DENV-3 isolates (from Mandera, Mombasa and Wajir) originated from those of Brazil, Paraguay and Peru, and were related to isolates from Pakistan, China and India. However, information on the potential of a given clade to transmit given viruses was not determined. Phylogenetic and epidemiological studies have shown that Dengue serotypes with more virulence can drive out virus strains of lesser epidemiological impact (Bravo *et al.*, 2014).

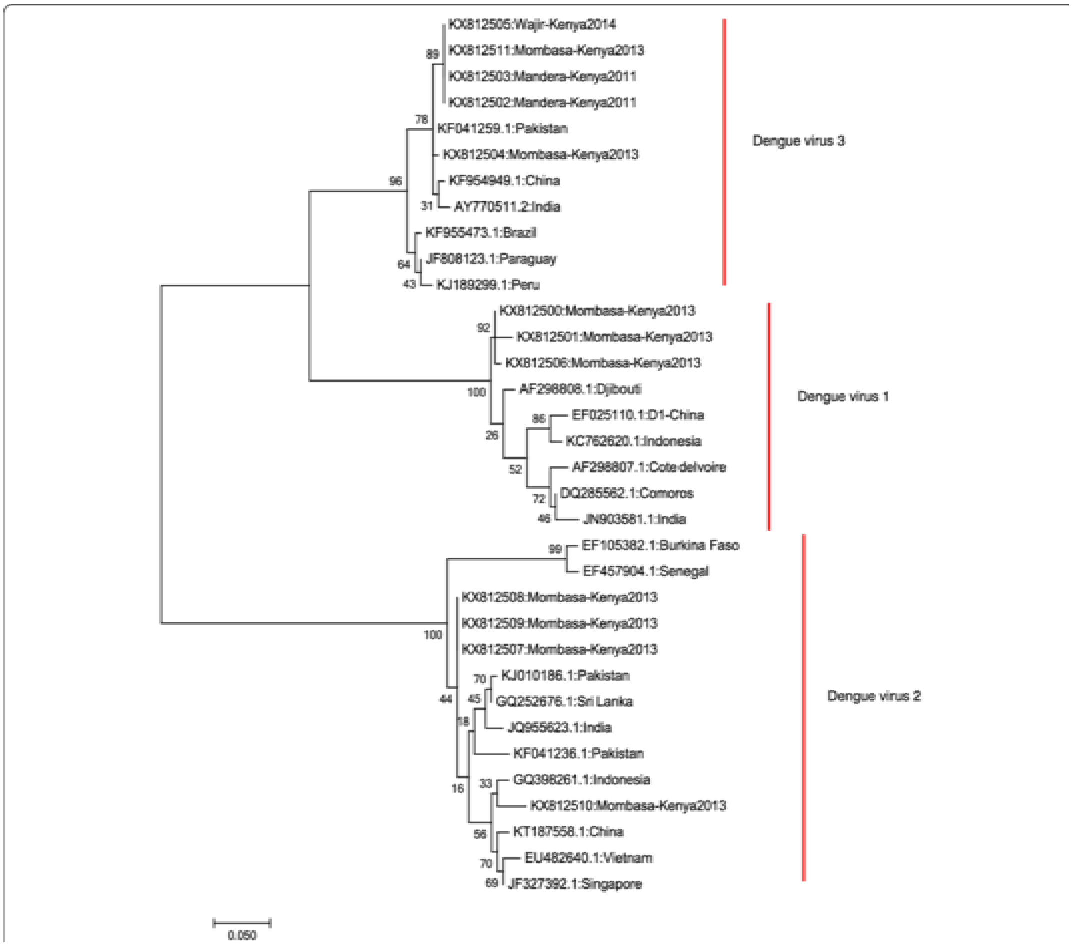


Figure 2.7: Phylogeny of isolates of Dengue virus {Adapted from (Konongoi *et al.,* 2016)}.

2.8 Genotypes of Chikungunya virus and their Global distribution

Chikungunya was first reported in Tanzania among the Makonde people between the years 1952 and 1953 (Zeller *et al.,* 2016). Since then, the virus has been reported within the tropical regions of Africa, America, Europe and Asia as shown in Figure 2.8: the virus is present in central and northwest America, west, central and southern Africa, southern Europe and south east Asia.

Three genotypes of this virus have been characterised: West African genotype (WAG), East Central South African (ECSA) genotype and Asian genotype (AG) (Lanciotti and Valadere, 2014). The Asian genotype has been reported in several areas around the globe: La Reunion Island in 2005 (Renault *et al.,* 2007), India in 2006 (Dash *et al.,* 2007), Northern Italy in 2007 (Moro *et al.,* 2010), south East France in 2010 (Grandadam *et al.,* 2011), New Caledonia in 2011 (Cao-Lormeau, *et al.*, 2014), Americas Island of St. Martin in 2013 (Leparc-Goffart, *et al.*, 2014), Kiribati and Cook Islands in 2015 (Roth *et al.*, 2014), south East Asia and Brazil in 2014 (Castro & Oliveira, 2015).

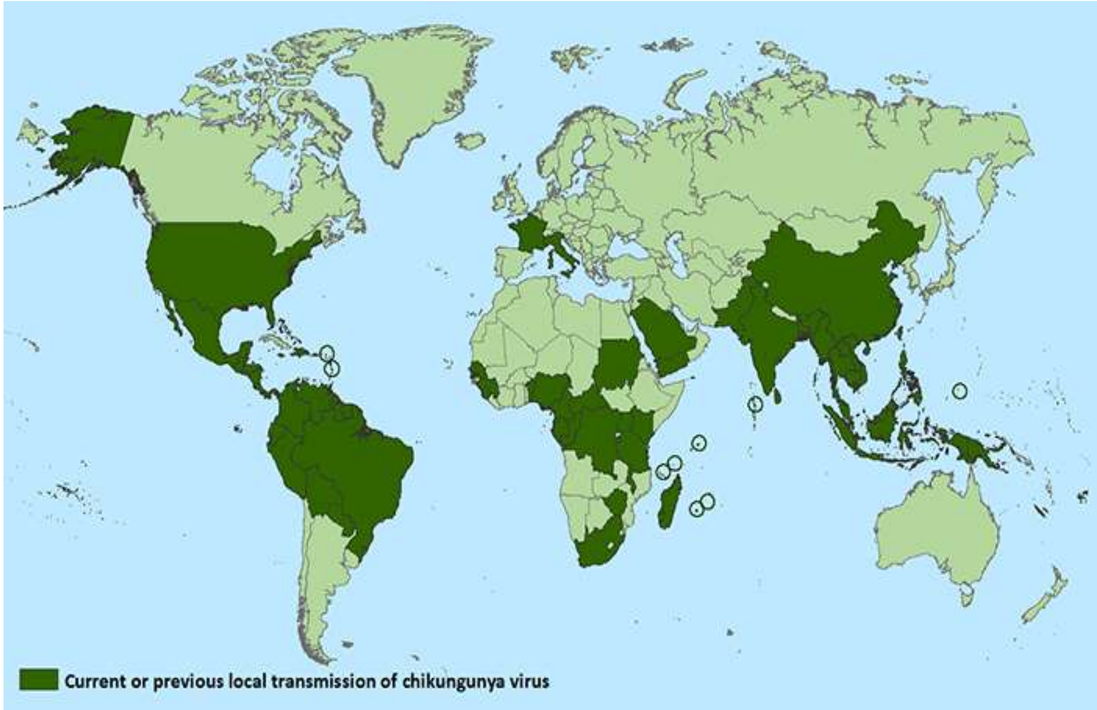


Figure 2.8: Global distribution of Chikungunya virus {Adapted from (Lanciotti and Valadere, 2014)}.

2.9 Epidemics of Chikungunya virus

Chikungunya virus was first isolated in Africa and has been reported in certain geographical regions with great health and economic effects (Kajeguka *et al.,* 2016). The virus has been reported in different corners of Africa, Middle East, Europe, America, India and Southeast Asia (Lanciotti & Valadere, 2014). The epidemics are due to human infections by both ECSA and Asian genotypes, either in isolation or simultaneously depending on the locality (Thiberville *et al.,* 2013b). In Africa, Chikungunya outbreaks have been reported in several African countries: Tanzania in 1952 (Kajeguka *et al.,* 2016), Comoros Island located off the coast of Tanzania in January 2005; Islands of Mayotte, Seychelles, La Reunion and Mauritius in 2005 (Sergon *et al.,* 2008; Vazeille, *et al.,* 2010). In Kenya, the virus was first reported in Lamu Island in 2004 during a maiden outbreak of Chikungunya virus infection (Sergon *et al.*, 2008; Njenga *et al.*, 2008; Zeller *et al.*, 2016). Recently, the virus was reported in the northern region of the county (Konognoi *et al.*, 2018). The maiden outbreak in Lamu involved about 75% of the entire Lamu population thus considered more catastrophic compared to other contemporary African Chikungunya outbreaks (Petersen *et al.*, 2016).

The ECSA genotype was reported to be responsible for the outbreaks in Kenya although vector and/ or entomological screening should be done in more sites (Njenga *et al.*, 2008). Chikungunya outbreaks are associated with changing weather conditions (rainfall and elevated temperatures) conducive for breeding of *Aedes* mosquitoes (Agha *et al.*, 2017). In urban centres, towns and cities, storage of domestic water in black containers due to water shortages and rationing provides ready breeding grounds for *Aedes* mosquitoes (Lutomiah *et al.*, 2016).

Seroprevalence of Chikungunya virus was reported at 0.97% (Ochieng *et al.*, 2015) and at 2.6% in people around lake or river basins in Kenya (Tigoi *et al.*, 2015). In the coastal region of Kwale, seroprevalence of the virus was reported at 6% (La Beaud *et al.*, 2015). These reports indicate active circulation of the virus within human and vector populations thus need for continuous surveillance and mapping of the virus and its respective vectors in Kenya.

2.10 Phylogeny of Chikungunya virus

Previous studies on phylogeny of alpha viruses reported existence of evolutionary relationship among isolates as shown in Figure 2.9 (identified isolates are highlighted in yellow): there was a close relationship between isolates from Kisumu and Naivasha. Similarly, isolates from Baringo were closely related to those from Garissa. Chikungunya virus was paraphyletic to Sindibis, Ockelbo, Babanki and Ndumu viruses.

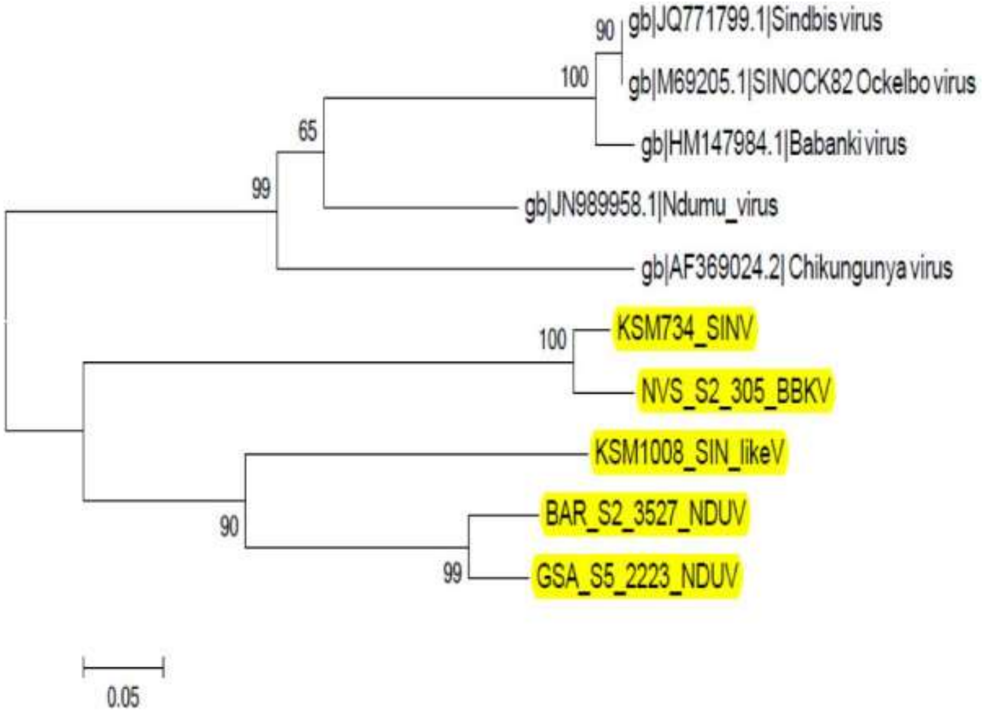


Figure 2.9: Phylogeny of isolates of Alphavirus {Adapted from (Ochieng *et al.*, 2013)}.

Phylogenetic analysis among genotypes of Chikungunya reported that the Asian genotype emerged from the ECSA sometime between 1879 and 1927 (Volk *et al.*, 2010). More so, there were indications that the Asian genotype was related to strains from Asia and the Caribbean (Yoon *et al.*, 2015). In Kenya, evolutionary studies on the ECSA genotype were conducted (Njenga *et al.*, 2008) as shown in Figure 2.10: the Kenyan isolates (highlighted in red) showed a close evolutionary relationship among themselves and to those from China and were clearly distinct with little variation between them.

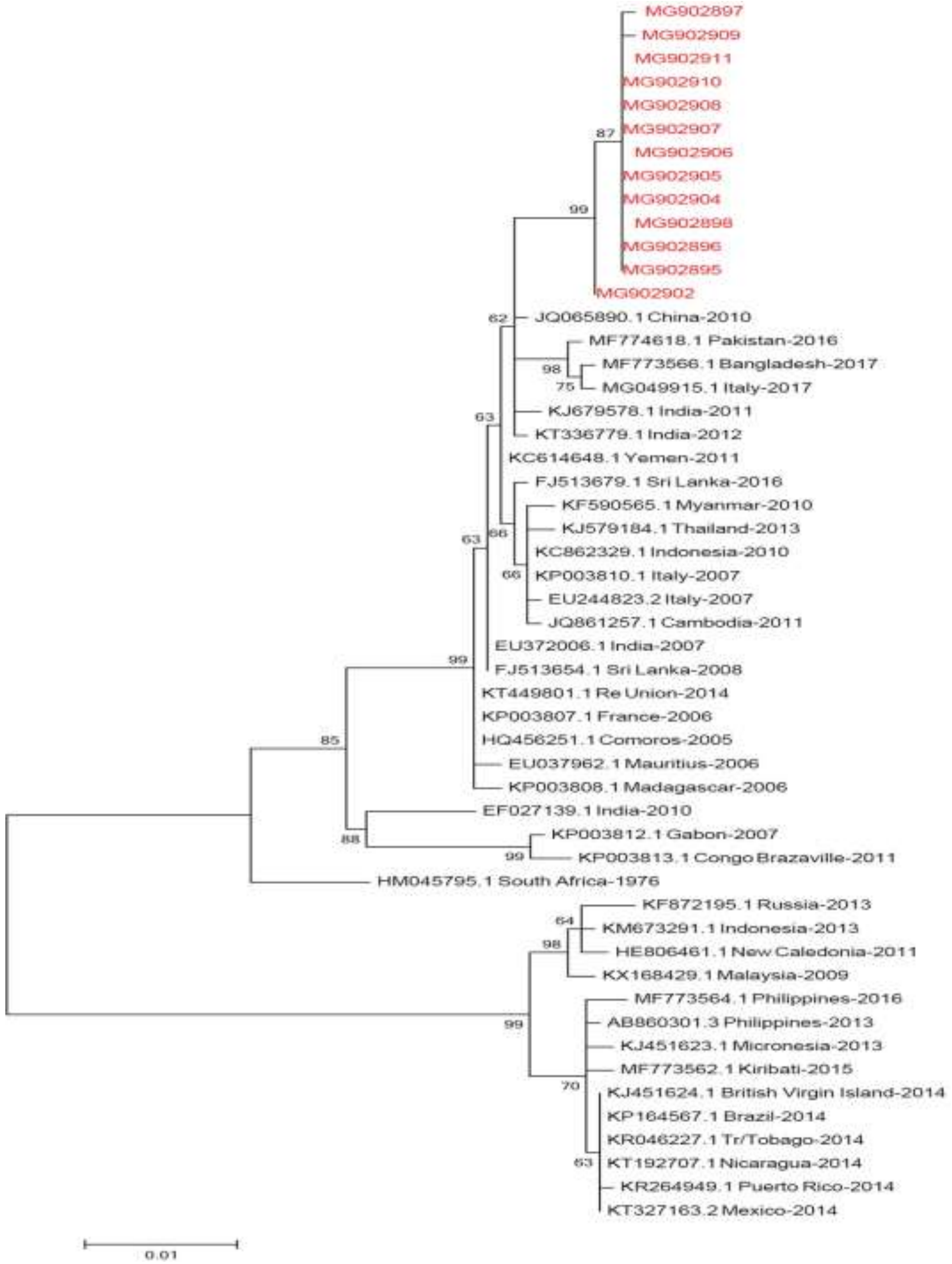


Figure 2.10: Phylogeny of isolates of Chikungunya virus {Adapted from (Konongoi *et al.*, 2018)}.

2.11 Co-infection of Arboviruses in *Aedes* mosquitoes

Aedes mosquitoes have been reported to transmit arboviruses in nature (da Costa *et al.,* 2017). The ability of a given *Aedes* species to carry multiple viral infections was earlier demonstrated in *Aedes albopictus*. The mosquito vector had the ability to become orally infected simultaneously with Dengue and Chikungunya viruses, and transmit them to human hosts via saliva during a blood meal (Vazeille *et al.*, 2010). Similar findings were reported for *Aedes aegypti* with Chikungunya and Zika viruses with a vector competence going up to 73% for Zika, 21% for Chikungunya and 12% of mosquitoes transmitting both viruses in one bite (Göertz, *et al.,* (2017).

Individual human beings have been reported to naturally acquire more than one virus at a time (Rückert *et al.,* 2017). The human co-infections could mean the vectors pick more than one virus during a blood meal (Le Coupanec *et al.,* 2017). *Aedes* mosquitoes could get co-infections of Dengue-2, Chikungunya and Zika viruses and potentially transmit all of them simultaneously (Rückert *et al.,* 2017). Furthermore, studies have demonstrated that mixed infection of Chikungunya and Dengue facilitates viral replication in *Aedes aegypti* thus increasing in their numbers before exit (Le Coupanec *et al.,* 2017). However, the question of vector preference by viruses had not been addressed.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sentinel study sites

Sentinel study sites were identified based on historical reports on seroprevalence and outbreaks of Dengue and Chikungunya fever (Chretien *et al.,* 2007; Njenga *et al.,* 2008; Sergon *et al.,* 2008; Sutherland *et al.,* 2011; Ellis *et al.,* 2015; La Beaud *et al.,* 2015; Mbaika *et al.,* 2016; Lutomiah *et al.,* 2016; Ngoi *et al.,* 2016; Konongoi *et al.,* 2016; Agha *et al.,* 2017). The sites were also selected due to favourable climatic conditions and ecology for breeding of *Aedes* mosquitoes.

The selected study sites were in the Counties of Lamu, Kilifi, Mombasa and Kwale along the Coastline of Kenya as shown in Figure 3.1. A total of 17 sentinel study sites from the 4 counties were selected randomly: Mpeketoni in Lamu County; Malindi, Watamu, Kilifi, Rabai, Mazeras in Kilifi County; Port Reitz, Tudor, Tononoka, Nyali, Shimo-la-tewa in Mombasa County; Kwale, Tiwi, Diani, Msambweni, Lunga lunga and Vanga in Kwale County. In Lamu, mosquito sampling was done in marine ecological zone and peri-urban sites. In Kilifi and Kwale, sampling was done in urban, marine and rural zones. In Mombasa, sampling was done in urban and marine ecological zones.

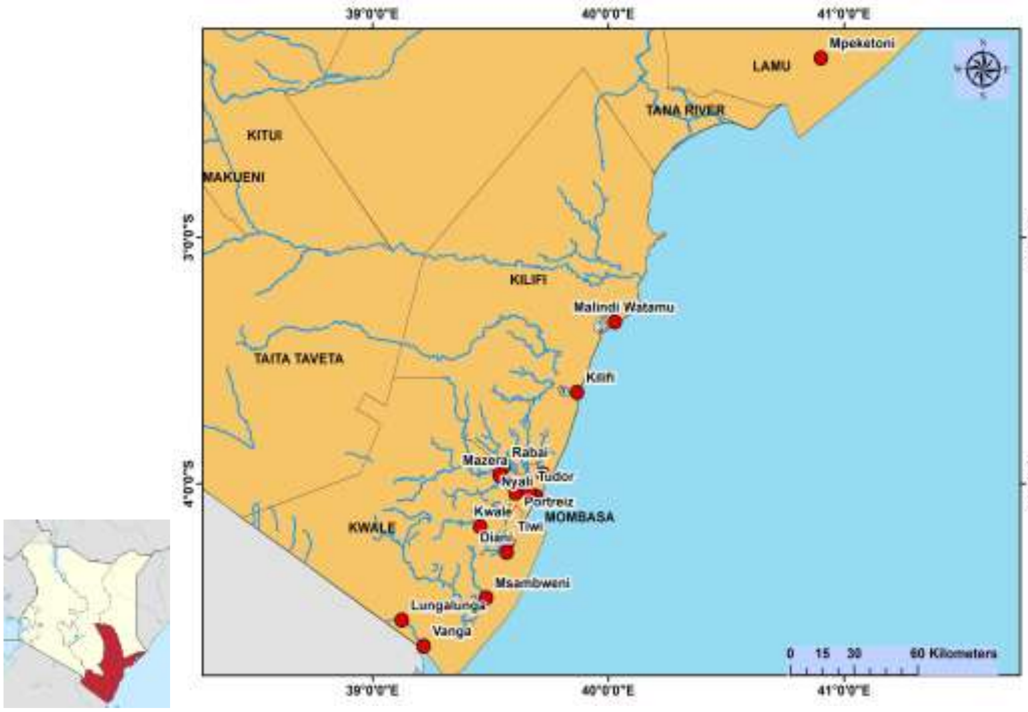
Lamu County lies on latitude 2˚16´10"S and longitude 40˚54´8"E with elevation of between zero and 50 metres above sea level (www.meteo.go.ke). The County experiences a mean rainfall of 417.15 mm, with more rain experienced between months of March to May and little rains experienced between the months of

October to December. Average temperature is at 27.3˚C with relative humidity of 80% (www.meteo.go.ke). Marine fishing is the main economic activity in this County.

Kilifi County lies on latitude 3˚37´49"S and longitude 39˚50´59"E with elevation of 10-24 metres (78 feet) above sea level (www.meteo.go.ke). The County experiences mean daily temperature of 30˚C with relative humidity of 82%. The County experiences two rain seasons: long rains in the months of March to May and short rain season in the months of October to December. Average rainfall is approximately 88.25 mm (www.meteo.go.ke). This is a County with various economic activities with fishing, livestock production and crop farming as the main drivers of the economy.

Mombasa County lies on latitude 4˚03´16"S and longitude 39˚39´48"E with elevation above the sea level at 20 metres (65 feet) (www.meteo.go.ke). The county experiences both hot and wet climate with long rains between the months of March to May, while short rains between the months of October to December. The average temperature is 26.3˚C with an average mean rainfall of 1072.7 mm and relative humidity of 77.6% (www.meteo.go.ke). The main economic activities in this area include tourism and fishing.

Kwale County lies on latitude 04.17489°S and longitude 039.45586°E with an elevation of 4 metres in Vanga to 387 metres above sea level in Kwale town. The County experiences what is described as tropical climate with relatively more rainfall experienced during long rain season and little rainfall during short rain season. The average temperature is 26˚C with an average mean rainfall of 1118 mm per year and relative humidity of 67% (www.meteo.go.ke). The main economic activities include tourism and fishing with little agriculture. Inhabitants of all the four Counties store water in tanks for domestic and agricultural use which provide readily breeding grounds for *Aedes* mosquitoes. More so, the climatic conditions (rainfall, humidity and temperature) described in each sentinel site are ideal for breeding of *Aedes* mosquitoes which are vectors for Dengue and Chikungunya viruses (Day, 2016).



(a) (b)

Figure 3.1: Study sentinel sites. (a) A map of Kenya indicating the coastal region. (b) Sentinel study sites on the Coastline of Kenya.

3.2 Study Design and Methodology

This was a cross sectional study which involved field and laboratory experiments as depicted in Figure 3.2: field work involved collection of adult *Aedes* mosquitoes in their natural habitat using Bio Gent (BG) sentinel traps (Biogents, Regensburg, Germany) and aspiration technique using Prokopack (John W. Hock, Gainesville, Florida, U.S.A). Laboratory experiments involved stunning, sorting and morphological identification of the mosquitoes. This was followed by extraction of RNA from pools of whole *Aedes* mosquitoes using Trizol®. RNA was subjected to both multiplex real time polymerase chain reaction (multiplex qPCR) and One-step reverse transcription (RT-PCR) for identification of *Aedes species* and respective viruses. Amplicons were subjected to Gel electrophoresis and purification. Purified amplicons were sequenced using Sanger method. Data analysis involved determination of proportions and prevalence of *Aedes* species, Dengue and Chikungunya viruses using R-statistics. Plotting of distribution maps for vectors and viruses was done using ArcGeographical information system (ArcGIS). Sequence files were used in plotting of phylogenetic trees using Molecular Evolution Genetic Analyser version 7.6 (Pevsner, 2015; Kumar *et al.*, 2016).

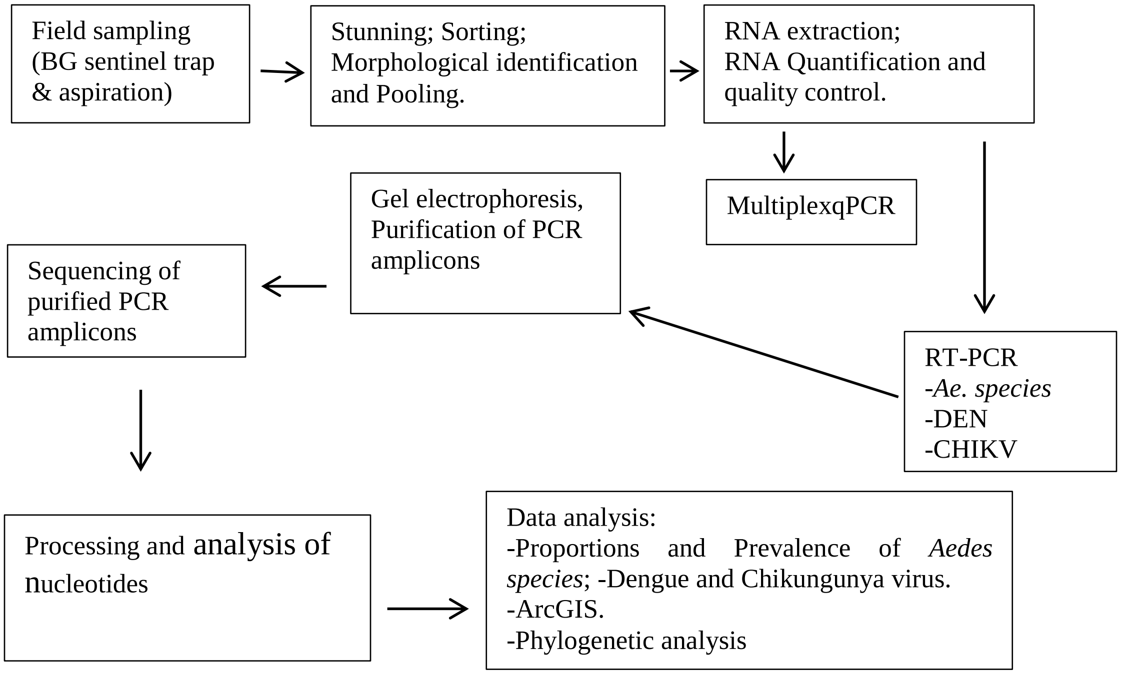


Figure 3.2 : Flow chart of study design and methodology

3.3 Identification, Spatial and Temporal distribution of *Aedes* mosquitoes

3.3.1 Entomological Sampling

Aedes mosquito sampling surveys were conducted in two different seasons: wet season (April to July and October to December) and dry season (January to March and August to September) for a period of two years. This was done to compare vector species, their proportions, their sex orientation, and their distribution and infection status by Dengue and Chikungunya viruses. Indoor and outdoor sampling of adult *Aedes* mosquitoes was by Bio gent sentinel traps (Biogents, Regensburg, Germany) and aspiration techniques as describe earlier by (Lutomiah *et al.*, 2016). Briefly, the BG traps were baited with solid carbon dioxide (dry ice) and were placed randomly in the sampling sites and houses to ensure good sample representation of the *Aedes* mosquito population. The BG traps were suspended at least 0.5 metres from the ground during outdoor sampling, while during indoor sampling, the traps were set near potential breeding sites like water storage tanks, jeri cans and buckets.

Prokopack aspirators (John W. Hock, Gainesville, Florida, U.S.A) were used for aspiration of mosquitoes. Outdoor aspiration was done from their resting and feeding sites: tyres, black tins, flower bushes, forests and homesteads. Indoor aspiration was done around water storage containers. Both BG trap and aspiration sampling was twice per day: at dawn between 0500hrs to 1000hrs and in the afternoon between 1500hrs to 1800hrs. All sampling sites were geo-coded for co-ordinates using global positioning system receiver (GPSR) for plotting of vector and viruses spatial and temporal distribution maps. All captured mosquitoes were transported live in net cages to the field sorting insectary.

3.3.2 Morphological identification of *Aedes* mosquitoes

In the sorting insectary, the live *Aedes* mosquitoes were stunned using dry ice as earlier described (Iwashita *et al.,* 2018). Briefly, the live mosquitoes were placed on a white filter paper in a petri dish full of dry ice for 5 minutes. The stunned mosquitoes were identified morphologically to their species level, physiological status and sex before sorting. Morphological identification of mosquito species was by stereoscopic microscope (WITec, Germany) as earlier described (Gillies and De Meillon, 1968) and according to manual "Mosquitoes of the Ethiopian Region" (Edward, 1941; Habarch, 1988; Reinert, 2000; Huang, 2002). *Aedes* *aegypti* s.l were further classified as either *Aedes aegypti aegypti* or *Aedes aegypti formosus* using scale pattern system as earlier described (McClelland, 1960). Briefly, *Aedes aegypti* s.l with any white scales on the first abdominal tergite was designated *Aedes aegypti aegypti*. If the first abdominal tergite was completely lacking the white scales then the individual was designated *Aedes aegypti formosus*.

3.3.3 Molecular identification of *Aedes* mosquitoes

Morphologically identification to species level was confirmed by molecular technique: One-step reverse polymerase chain reaction (RT-PCR) using specific forward and reverse primers (Invitrogen) indicated in Table 3.1. Only the unfed and gravid mosquitoes were utilised in this analysis. The female blood-fed were excluded to avoid contamination of the virus which could be in the blood meal. The samples were grouped in pools of 20 and cryopreserved in 1.5 ml cryogenic vials at -80˚C for RNA processing to avoid its degradation.

Table 3.1: Primers used in identification of *Aedes* mosquitoes

Target	**Primer name**	**Nucleotide sequences (5' to 3')**	**Polarity**	**Product (bp)**
Mosquito RNA marker (UP)	Act-2F	ATGGTCGGYATGGGNCAGAAGGACTC	Forward	683
	Act-8R	GATTCCATACCCAGGAAGGADGG	Reverse	
Ae. aegypti s.l	18SFHIN	GTAAGCTTCCTTTGTACACACCGCCCGT	Forward	550
	CP16	GCGGGTACCATGCTTAAATTTAGGGGT		
	Aeg.r1	TAACGGACACCGTTCTAGGCCCT	Reverse	
Ae. tricholabis	UV	TGTGAACTGCAGGACACAT	Forward	
Ae. pembaensis	PEM	GCATCGATGGGTTAATCATG	Reverse	405
Ae. ocharaceous	OCH	CAAGCCGTTCGACCCTGATT	Reverse	501
Ae. albicosta	ALB	CCTGGCCAGTGGCCAAAT	Reverse	
Ae. fulgens	FUL	GTGCACACCACTGAATT	Reverse	
Ae. mcntoshi	MCN	CTGATGCACTGGCCTCAAAG	Reverse	
Ae. fryeri	FRR	TCAACCGCCGTGCGTG	Reverse	
ND4	ND4sb+	TGATTGCCTAAGGCTCATGT	Forward	344
	ND4sb-	TTCGGCTTCCTAGTCGTTCAT	Reverse	

3.3.4 RNA extraction

Extraction of RNA and determination of its quantity and quality was done as follows:

a) Homogenization: Pools of *Aedes* mosquitoes were homogenized as described by Chomczynski, (1995). Briefly, this was done by a mortar and pestle. 1 ml of Trizol® was added into the pestle with the mosquito pools and ground thoroughly and vortexed (FisherbrandTM ZX4 IR Vortex mixer, Fisher Scientific, Bishop

Meadow Road, Loughborough, Leicestershire, LE11 5RG, UK) one minute. An aliquot of the solution was transferred to eppendorf tubes and left in Trizol® at room temperature for five minutes.

b) Phase Separation: A 0.2 ml of chloroform was added to the samples which was then capped and vortexed for 15 seconds. Incubation of the samples was done at room temperature for 2-3 minutes. Centrifugation using high performance centrifuge (Labnet international, Inc.) was done at 12,000 rpm for 15 minutes at 8˚C.

c) RNA Precipitation: Centrifugation resulted in three phases visible within the tube: an aqueous phase (upper), an organic phase (lower) and an interphase. The aqueous phase at the top was transferred into a fresh tube and 0.5 ml of isopropanol added. The tubes were incubated at room temperature for 10 minutes followed by centrifugation at 12,000 rpm for 10 minutes at 8˚C.

d) RNA wash and Re-suspension: Following centrifugation, the supernatant was removed and RNA pellet washed with 1 ml of 75% ethanol. This was vortexed followed by centrifugation at 7,500 rpm for 5 minutes at 8˚C. The supernatant was removed and the remaining ethanol air dried for 2-3 minutes. The pellet was eluted in elution buffer containing sodium azide and vortexed for a minute. Tubes were transferred to a digital dry bath at 60˚C for 15 minutes before placing them on ice for quantity and quality analysis.

e) Quantification and Quality control: Quantity and quality of extracted RNA was determined using a Nano drop spectrophotometer (N-1000) (Thermo-Fisher Scientific, Wilmington, DE). Sample concentration and purity was determined using 1.5 µl micro cuvette with ratio of absorbance at 260 nm and 280 nm. The total RNA was eluted in 50 µl of sterile distilled water.

3.3.5 Identification of *Aedes species*

One-step reverse transcription polymerase chain reaction (One-step RT-PCR) was done on 10 µl of the extracted RNA using EcoDry Premix (Random hexamers) (Clontech laboratories, Inc., Mountain view, CA, USA), AccuPower^{TM} PCR Premix kit (Bioneer Co., Daejon, Korea) with *Aedes* specific primers (Invitrogen) indicated in Table 1. Briefly, 10 µl of the RNA was mixed with 2 µl of 50ng/ µl random hexamer primer and 1 µl of 10 mM deoxynucleotide solution (dNTPs) in a 200 µl PCR tube. This was incubated in a thermo cycler (Applied Biosystems, Foster City, Califonia, USA) for 5 minutes at 65°C and immediately chilled for 1 minute at 4°C. 4 µl of 5x first strand buffer (Invitrogen), 1 µl of 0.1 M DTT, 1 µl of RNAse (40 U/ µl) and 1 µl of Superscript III reverse transcriptase (200U/ µl) were added into the PCR tube. The mixture was incubated in a thermo cycler (Applied Biosystems, Foster City, Califonia, USA) for 5 minutes at 25°C, 50 minutes at 50°C and 15 minutes at 70°C. A total of 20 µl cDNA was generated. 2 µl of the PCR amplicons were subjected to gel electrophoresis using 1.5% agarose gel with respective positive controls for the *Aedes species* stained with ethidium bromide and visualised under Accuris E3000 ultra violet trans-illuminator (Benchmarks Scientific, Horsham, PA).

3.3.6 Spatial and temporal distribution of *Aedes* mosquitoes

Spatial and temporal distribution of *Aedes* mosquitoes was determined for a period of two years (2015 to 2017) during dry and wet seasons to estimate risk of arboviral outbreaks. Coordinates of the sentinel study sites were recorded using Global Positioning System Receiver (GPSR) for plotting of maps using ArcGeographical information system (ArcGIS) to determine dynamics of vectors in relation to dry and wet seasons along the coastline of Kenya.

3.4 Phylogeny of *Aedes* mosquitoes

Phylogeny of *Aedes* mosquitoes was based on mitochondrial Nicotinamide Adenine dinucleotide dehydrogenase sub unit 4 genes (mtNAD4 gene). 2.5 µl of RNA was used in amplification of the mtNAD4 gene using primers shown in Table 3.1. Optimisation of the primers was on a gradient thermal cycler at an annealing temperature of 56°C. Amplification of the 344 base pair fragment of ND4 gene was by five minutes denaturation at 96°C. This was followed by 35 cycles of amplification with 40 seconds at 94°C, 40 seconds at 56°C and 40 seconds at 72°C then a final extension step for five minutes at 72°C. The amplicons were subjected to gel electrophoresis using 1.5% agarose gel stained with ethidium bromide and visualised under Accuris E3000 ultra violet trans-illuminator (Benchmarks Scientific, Horsham, PA). The amplicons were excised and recovered from the gel using MinElute PCR purification kit (Qiagen, Valencia, CA) and sequenced in both directions using Sanger high-throughput technique as described by Sanger, *et al.,* (1977).

3.5 Phylogeny of Dengue and Chikungunya viruses

3.5.1 Identification of Serotypes of Dengue virus

Identification of Dengue virus was based on amplification of the target viral genes (E/NSP1 and NSP5) in RNA using multiplex qPCR as described by Kuno *et al.,* (1998). This was a step wise procedure: Samples were first tested for viruses using a panel of general flavivirus family primers (Appendix 5). Optimisation of primers was done on a gradient thermal cycler for the best annealing and denaturing temperatures using an RNA library extracted from previously cultured Dengue virus. The optimum PCR conditions were then used for detecting Dengue virus in the collected mosquito samples. Samples testing positive for flavivirus were further tested with consensus primers for Dengue virus. These primers were DEN-F and DEN-CR and they target the E/NSP1 junction of the virus genome.

Therefore, the presence of Dengue virus was detected using the CDC DENV 1-5 qPCR assay kit using SuperScript^{TM} III Platinum One-step qPCR system (Invitrogen, Carlsbad, Califonia, USA) using probes in Appendix 5. This was in a total reaction volume of 25 µl containing 5 µl of RNA, 4.5 µl nuclease free water, 12.5 µl 2xRT-PCR buffer, 1 µl of 25xRT-PCR enzyme mix, 0.8 µl of 20 picomoles forward primer and reverse primer, 0.4 µl of 5 picomoles of the probe. Reactions were run in 96 well plates in the ABI 7500 FAST Dx thermocycler (Applied Biosystems, Foster City, Califonia, USA) under the cycling conditions of 45°C for 10 minutes, 95°C for 10 minutes, 45 cycles of 95°C for 15 seconds and

55°C for 1 minute. Analysis of the displayed curves identified positive samples for various serotypes of Dengue (Appendix 4).

Only samples testing positive with Dengue consensus primers were further tested for the four Dengue serotypes using appropriate primers in Table 3.2. One step reverse transcription polymerase chain reaction (One-step RT-PCR) was done on samples positive for Dengue virus in the multiplex qPCR for confirmation. The amplification was done in a 25 µl reaction mixture consisting of:12.5 µl of Amplitaq Gold 360 PCR master mix (Applied bio systems, USA), 0.5 µl of 50 picomoles each of forward and reverse primer, 2 µl of cDNA and 9.5 µl of Diethyl pyrocarbonate (DEPC) treated water to top up to 25 µl (Norgen Biotek, Canada). The mixture was incubated at 50°C for 20 minutes, 95°C for 5 minutes, followed by 40 cycles of 95°C for 15 seconds, 60°C for 30 seconds, and 70°C for 1 minute, and a final extension for 10 minute at 72°C. Electrophoresis of the amplicons was by 1.5% agarose gel in 1% Tris-borate EDTA buffer stained with ethidium bromide. The band pattern was visualised using Accuris E3000 ultra violet trans-illuminator (Benchmarks Scientific, Horsham, PA). Amplicons from the gel was excised and recovered using the MinElute PCR purification kit (Qiagen, Valencia, CA) for processing and sequencing.

Table 3.2: Primers for identification of serotypes of Dengue virus

Primer	**Gene/ protein target**	**Primer sequence (5’ to 3’)**	**Position**
FU1	NSP5	TACAACATGATGGGAAAGAGAGAGAA	9007-9032
CFD3	NSP5	GTGTCCCAGCCGGCGGTGTCATCAGC	9308-9283
DEN-F	E/NS1	TCAATATGCTGAAACGCGCGAGAAACCG	38-65
DEN-CR	E/ NS1	TTGCACCAACAGTCAATGTCTTCAGGTTC	455-483
TS1-R	NSP5	CGTCTCAGTGATCCGGGGG (DEN-F and TS1)	
TS2-R	NSP5	CGCCACAAGGGCCATGAACAG (DEN-F and TS2)	
TS3-R	NSP5	TAACATCATCATGAGACAGAGC(DEN-F and TS3)	
TS4-R	NSP5	CTCTGTTGTCTTAAACAAGAGA(DEN-F and TS4)	
D5-F	NSP5	TCAATATGCTGAAACGCGHGAG	132-153
D5-R	NSP5	GCGCCTTCNGNNGACATCCA	764-783

3.5.2 Identification of Genotypes of Chikungunya virus

Identification of Chikungunya virus was based on amplification of the target viral genes E1 and NSP4 in the RNA using multiplex qPCR with AgPath-ID One-step RT-PCR kit (Applied bio systems, Carlsbad, Califonia, USA) using similar method described by Lanciotti *et al.,* (1992). Presence of alphaviruses was screened in all samples using a panel of general alphaviruses probes and primers indicated in Appendix 5. This was in a total reaction volume of 25 µl containing 5 µl of RNA, 4.5 µl nuclease free water, 12.5 µl 2xRT-PCR buffer, 1 µl of 25xRT-PCR enzyme mix, 0.8 µl of 20 picomoles forward primer and reverse primer, 0.4 µl of 5 picomoles of the probe. Reactions were run in 96 well plates in the ABI 7500 FAST Dx thermo cycler (Applied Bio systems, Foster City, Califonia, USA) under the cycling conditions of 45°C for 10 minutes, 95°C for 10 minutes, 45 cycles of 95°C for 15 seconds and 55°C for 1 minute. Analysis of the displayed curves identified positive samples for alpha viruses (Appendix 4).

Samples testing positive for alpha viruses were further tested with conventional primers for respective genotypes of Chikungunya virus in Table 3.3 for. Samples were subjected to One-step RT-PCR using a volume of 20.2 µl: 1 µl of RNA, 4 µl of 5 x reaction buffers, 1.8 µL of 25 mM MgCl2, 1.8µl of 10µM dNTP, 0.5µl of 20µM of each primers, 0.3 µl of Go-Tag Flex DNA polymerase, and 10.3 µl of nuclease free water (Norgen Biotek, Canada). Incubation was set at temperature of 95°C for 2 min; 40 cycles of 30 seconds denaturation at a temperature of 95°C; annealing at 55°C for 30 seconds; elongation at 72°C for 1 minute and final extension at 72°C for 5 minutes. The amplified products were subjected to gel electrophoresis on 1.5% agarose gel with 1% Tris-borate EDTA buffer stained with ethidium bromide. The fragments on the gel were visualised under Accuris E3000 ultra violet trans-illuminator (Benchmarks Scientific, Horsham, PA). The cDNA from the gel was excised and recovered using the MinElute PCR purification kit (Qiagen, Valencia, CA) for processing and sequencing.

Table 3.3: Primers for identification of Chikungunya virus

Primer	**Gene/ protein target**	**Primer sequence (5’ to 3’)**	**Position**
VIR 2052 F	NSP4	TGGCGCTATGATGAAATCTGGAATGTT	6971-6997
VIR 2052R	NSP4	TACGATGTTGTCGTCGCCGATGAA	7086–7109
CHIKV-F	E1	CGTGGTGTACAAAGGTGACG	10524
CHIKV-R	E1	ACG CCG GGTAGTTGACTATG	11170
CHIKWa	NSP1	GGCAAACGCAGTGGTACTTCCT	295-316
CHIKAs	NSP1	GGCAGACGCAGTGGTACTTCCT	295-316
CHIKECSa	NSP1	TGATCCCGACTCAACCATCCT	234-254

3.5.3 Nucleotides Sequencing

Gene products were sequenced using Sanger high-throughput technique as described by Sanger, *et al.,* (1977). Briefly, purified cDNA was sequenced using ABI-PRISM 3130 Genetic Analyser (Applied 42 Bio systems, Foster City, CA). The DNA strands which were complementary to the mother DNA strand (template strand) were assembled. Sequencing involved incorporation of deoxynucleoside triphosphates (dNTPs) and modified dideoxynucleoside triphosphates (ddNTPs) for strand elongation. The ddNTPs were modified in terms of their chemical structure and labelled with a fluorescent biomolecules containing inhibitors of the phosphodiester bond formation. This resulted into inhibition of DNA polymerase from elongating the DNA strand whenever a ddNTP was incorporated. The amplified gene products were separated using capillary electrophoresis. During electrophoresis, the amplified gene fragments moved through a gel-like matrix at different velocity depending on their gene size which was determined by the number of base pairs. The four chemically altered ddNTPs adopted different fluorescent labelled biomolecules. The fluorescence emitted from the respective excited fluorescent dye gave the identity of the nucleotide in the original cDNA template.

3.6 Data Analysis

Data on *Aedes* species collected was entered and cleaned in STATA 13. The semi-processed data in STATA was saved as comma separated values (csv) extension and transported to R. Proportions of *Aedes* species, sex orientation, and their viral (Dengue and Chikungunya) infection rates were determined in relation to dry and wet season. Fisher's least significant difference method for t-statistical test was used to compare proportions and prevalence of vectors and viruses among the Counties. Spatial and temporal maps for identified *Aedes* species, Dengue and Chikungunya viruses were plotted using ArcGeographical information system.

Generated nucleotide sequences were used for phylogenetic analysis. DNAbaser v.3.0 (http://www.dnabaser.com/articles/SNP) was used for editing bad calls in the raw chromatogram file generated from sequencing the forward and reverse strands. Deletion of the generated sequences of primers was done from the 5' and 3' ends. The sequences were subjected to Basic Local Alignment Tool (BLASTn) and GenBank database to compare them with available sequences and confirm the identity of the isolates. Formatting of the retrieved sequences compatible with alignment programs and identification of correct reading frame for each sequence was done using the translation program at http://us.expasy.org/tools/dna.html.

The sequences were aligned for identification and removal of duplicate sequences using Clustal Omega v1.2.1, scored in T-coffee (http://tcoffee.crg.cat/) and viewed in jalview http://www.jalview.org/ (Sievers and Higgins, 2014). The sequences were manually adjusted in Se-Al software according to DNA sequence alignments for preservation of codon homology as described by (Rambaut, 1996). Columns with more than one percent of gaps were removed from the alignment using trimAl v1.4.rev6 (Capella-Gutierrez *et al.*, 2009). Using pmodeltest v1.4, Maximum likelihood trees were inferred using Randomised Accelerated Maximum Likelihood (RAxML) version 8.1.20 ran with model GTR+GAMMA+I for selecting the best-fit model for the maximum likelihood analyses and plotting of phylogenetic trees using interactive tree of life (https://itol.embl.de/) (Stamatakis, 2014). Branch supports were computed out of 1000 bootstrap replicates based on the Tamura Nei model (Tamura, 1993).

CHAPTER FOUR

RESULTS

4.1 Spatial and temporal distribution of *Aedes* mosquitoes along the Coastline of Kenya

4.1.1 Composition and Proportions of *Aedes* mosquitoes along the Coastline of Kenya

A total of 37,220 *Aedes* mosquitoes were collected using BG trap and Prokopack aspiration technique in 17 sentinel sites along the Coastline of Kenya during dry and wet seasons. 58% (21,438 *Aedes* mosquitoes) were collected during wet season (Appendix 3). The species of *Aedes* mosquitoes identified were: *Aedes aegypti s.l*, *Aedes mcnitosh*, *Aedes pembaensis*, *Aedes ochraceus*, *Aedes tricholabis*, *Aedes albicosta*, *Aedes fulgens*, and *Aedes fryeri* (Figure 4.1). Sub-species of *Aedes aegypti s.l* were identified as *Aedes aegypti formosus and Aedes aegypti aegypti*. *Aedes aegypti aegypti* was identified for the first time in this study along the Coastline of Kenya. The composition and proportions of these *Aedes* species along the Coastline of Kenya did not differ significantly between seasons (M=2,068, SD=3,520), t_8=0.03, p>0.001. However, the proportion of *Aedes aegypti formosus* was significantly the highest in both seasons (*M*=64, *SED*=20.4), *t*(27)=41.86, *p*=0.025.

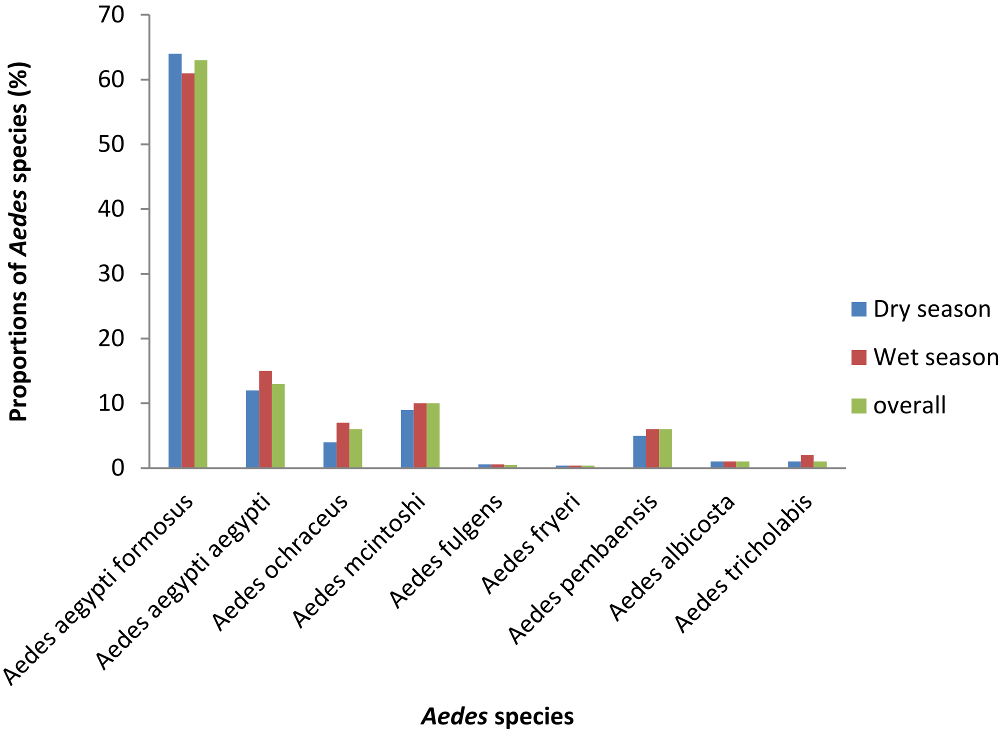


Figure 4.1: Composition and proportions of *Aedes* species sampled during dry and wet seasons along the Coastline of Kenya. *Aedes aegypti aegypti* was isolated for the first time along the Coastline of Kenya.

4.1.2 Proportions of sex of *Aedes* mosquitoes along the Coastline of Kenya

Although on average, more males (53%=19,651mosquitoes) than females (47%=17,569) were collected in both seasons as shown in Figure 4.2. However, the proportions of sex of *Aedes* mosquitoes along the Coastline of Kenya did not differ significantly between and within seasons, (M=2,068, SD=3,587), t_8=0.315, p>0.001.

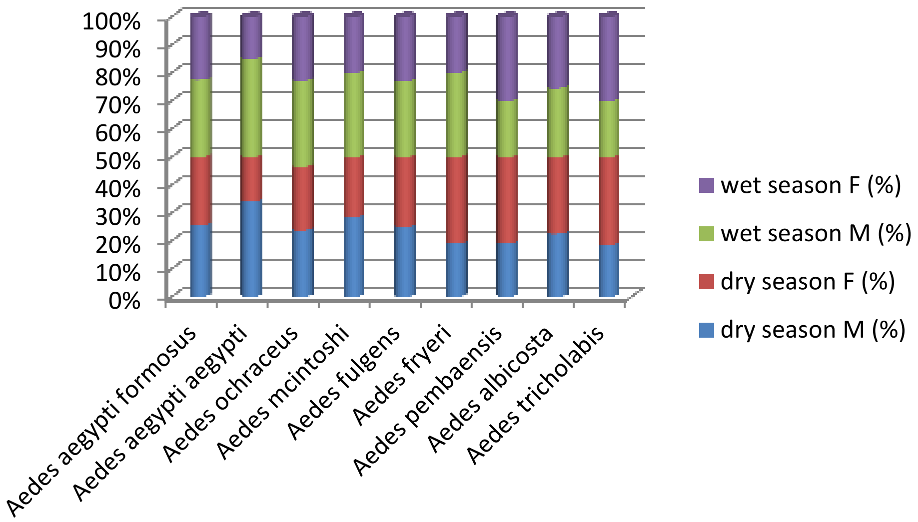


Figure 4.2: Proportions of sex of *Aedes* species along the Coastline of Kenya during dry and wet season.

4.1.3 Prevalence of *Aedes* mosquitoes along the Coastline of Kenya

On average, Kilifi County had the highest prevalence of *Aedes* mosquitoes at 31% as shown in Figure 4.3 and Appendix 3. The prevalence of *Aedes aegypti formosus* was significantly higher across all counties, *p*=0.025. *Aedes fulgens* and *Aedes fryeri* were isolated in Mombasa County only. In addition, the population of *Aedes tricholabis* in Kwale, Mombasa and Kilifi were significantly higher than that from Lamu, *p*=0.025.

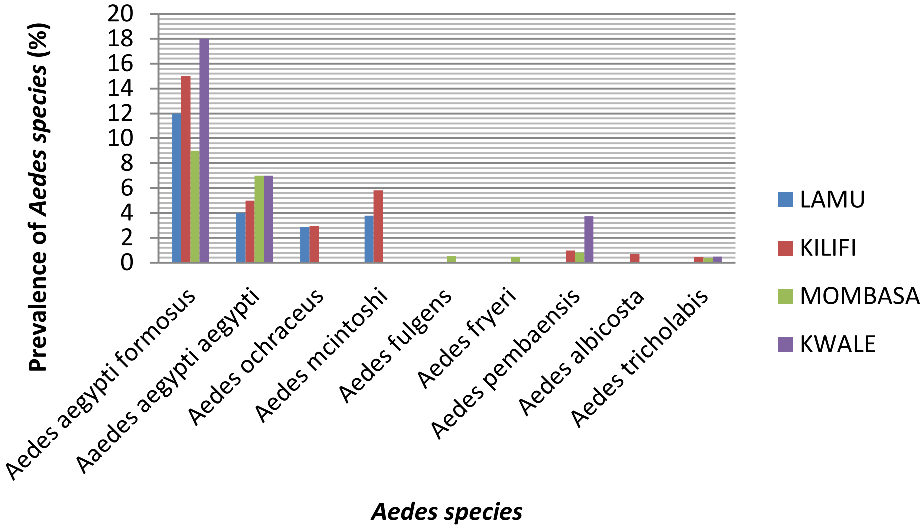


Figure 4.3: Prevalence of *Aedes* species along the Coastline of Kenya. *Aedes aegypti aegypti* and *Aedes aegypti formosus* were present in all Counties.

4.1.4 Distribution of *Aedes* mosquitoes along the Coastline of Kenya

Aedes aegypti formosus and *Aedes aegypti aegypti* were distributed along the entire Coastline of Kenya as shown in Figures 4.4 and 4.5. However, these two *Aedes* species were not found in the mangrove ecosystems of Watamu, Tudor and Vanga during dry and wet seasons. *Aedes ochraceus* and *Aedes mcintoshi* were only found in the northern parts of the Coastline: Kilifi, Malindi and Lamu. *Aedes fulgens* and *Aedes fryeri* were only found in Mombasa: Nyali and Port Reitz respectively. *Aedes pembaensis* and *Aedes tricholabis* were only found in marine mangrove ecosystems of Watamu, Tudor, Msambweni and Vanga. *Aedes albicosta* was only present in Kilifi and Malindi areas during both seasons.

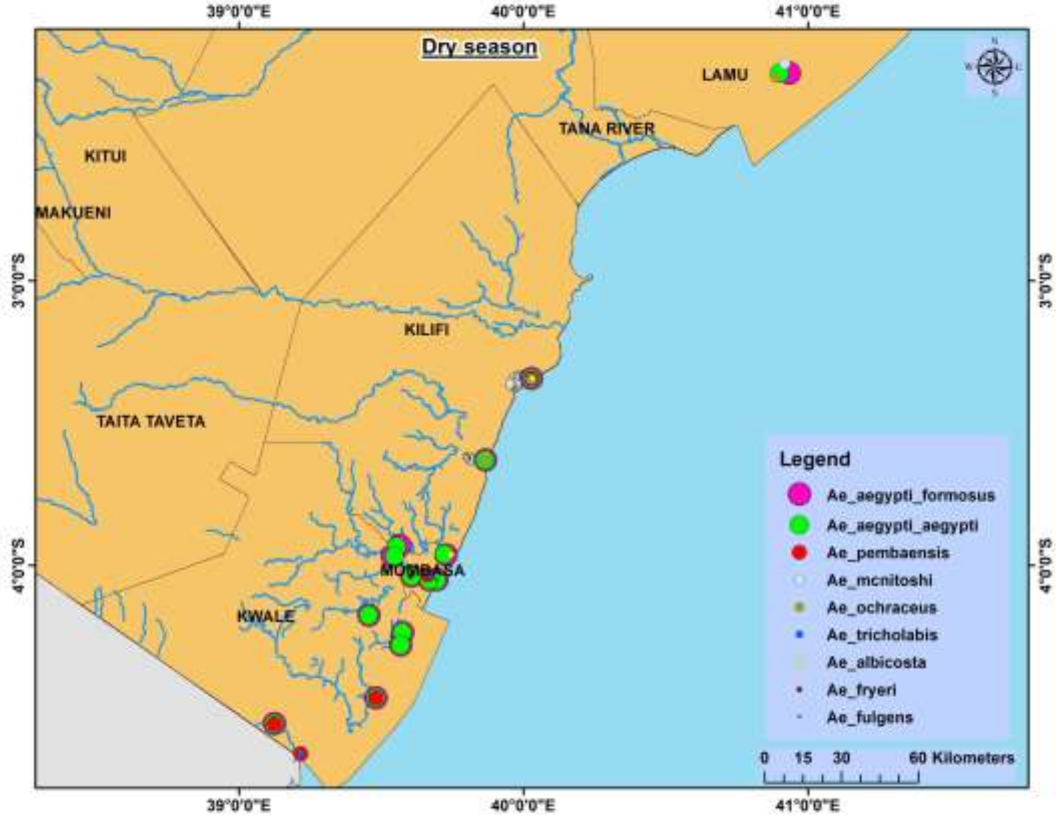


Figure 4.4: Distribution of *Aedes* mosquitoes along the Coastline of Kenya during dry season.

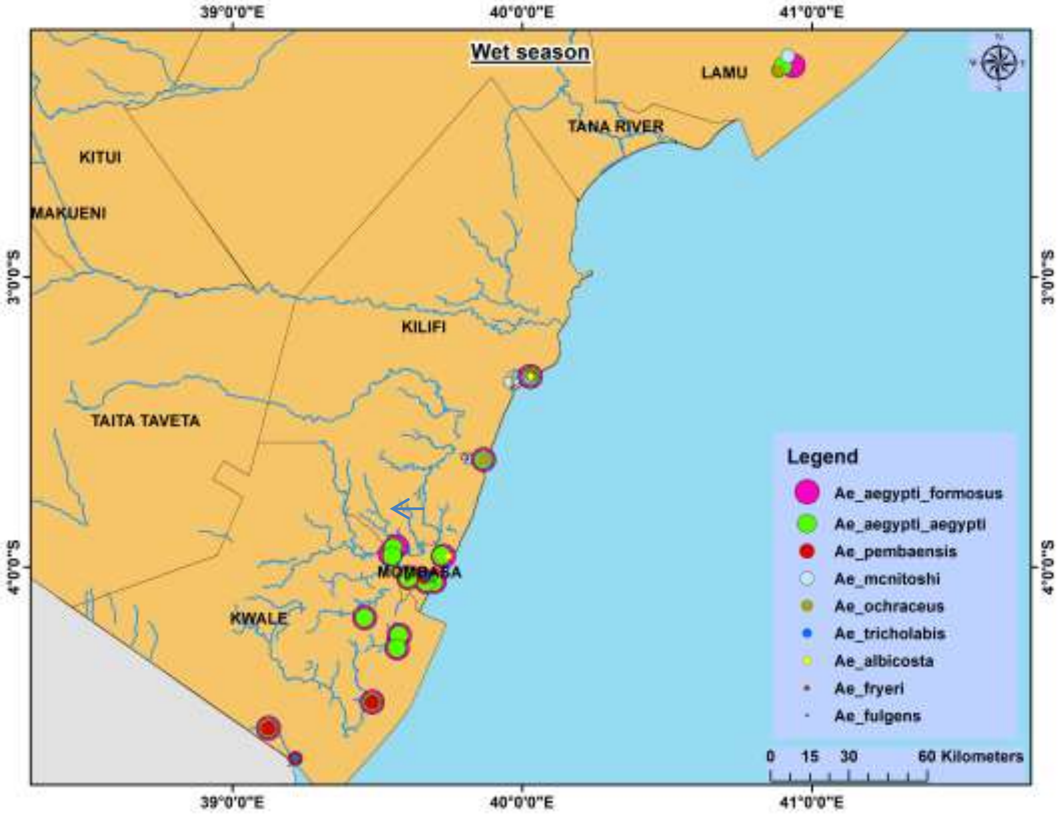


Figure 4.5: Distribution of *Aedes* mosquitoes along the Coastline of Kenya during wet season.

4.2 Phylogeny of *Aedes* mosquitoes along the Coastline of Kenya

The mitochondrial nicotinamide adenine dehydrogenase sub unit-4 gene (mtDNA NAD4) sequences of the eight *Aedes* species identified in this study were used to estimate their evolutionary relationships (Figure 4.6). A total of 21 new haplotypes of *Aedes* species were identified with haplotypes of *Aedes mcintoshi*, *Aedes albicosta* and *Aedes ochraceous* formed an orthologous gene group which is paraphyletic to the other *Aedes* species. *Aedes albicosta* and *Aedes ochraceous* from Kilifi formed a cluster. *Aedes ochraceous* and *Aedes mcintoshi* from Lamu formed a monophyletic group.

Haplotypes of *Aedes pembaensis*, *Aedes tricholabis*, *Aedes fulgens* and *Aedes fryeri* formed an orthologous gene group with two monophyletic sister groups (*Aedes pembaensis* and *Aedes tricholabis*) and (*Aedes fulgens* and *Aedes fryeri*). Members of *Aedes aegypti* s.l formed an orthologous gene group with *Aedes aegypti formosus* and *Aedes aegypti aegypti* clustering in two distinct clades. Haplotypes of *Aedes* mosquitoes from Kilifi were closely related to those from Lamu. Similarly, the haplotypes from Kwale were closely related to those from Mombasa.

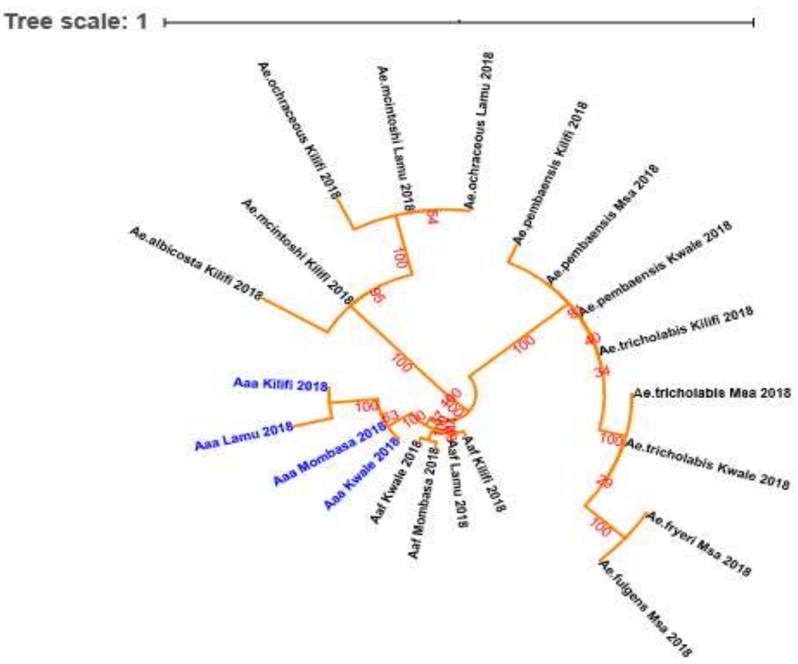


Figure 4.6: Phylogenetic relationships among *Aedes* mosquitoes sampled along the Coastline of Kenya. Tree based on the RAxML algorithm under the Tamura-Nei genetic distance model. Bootstrap values for 1000 replicates are indicated at the major branch points. Haplotypes highlighted in blue were identified for the first time along the Coastline of Kenya.

Phylogeny of the new haplotypes of *Aedes* mosquitoes along the Coastline of Kenya were compared with those previously identified in Kenya and other parts of the world (Figure 4.7).

*Aedes mcintoshi*_Kilifi_2018, *Aedes ochraceous*_Lamu_2018, and *Aedes albicosta*_Kilifi_2018 formed an out group in relation to all other *Aedes* species. All the new haplotypes were in derived clades of the in-group especially if compared to haplotypes from Senegal in West Africa (DQ176836), Brazil (AY906853), Mexico (JX297259) and Uganda (DQ176838). *Aedes tricholabis, Aedes pembaensis, Aedes fryeri, Aedes fulgens* formed an orthologous gene group. *Aedes aegypti formosus* from Kwale and Mombasa formed a monophyletic sister group and were closely related to haplotypes previously identified in Kenya (EU446267 and EU446273). In addition, the new isolates of *Aedes aegypti formosus* were closely related to haplotype JQ926721 from Thailand and to the global haplotype AF203356. *Aedes aegypti formosus* haplotypes from Lamu and Kilifi were closely related to EU446271 which was previously reported in Kenya and to that from Senegal DQ176836. All *Aedes aegypti aegypti* were in the same cluster and were closely related to haplotypes from Uganda_DQ176838, Mexico_JX297259 and Brazil_AY906853. *Aedes malayensis*_KY420820 from Singapore formed an out group with respect to this cluster.

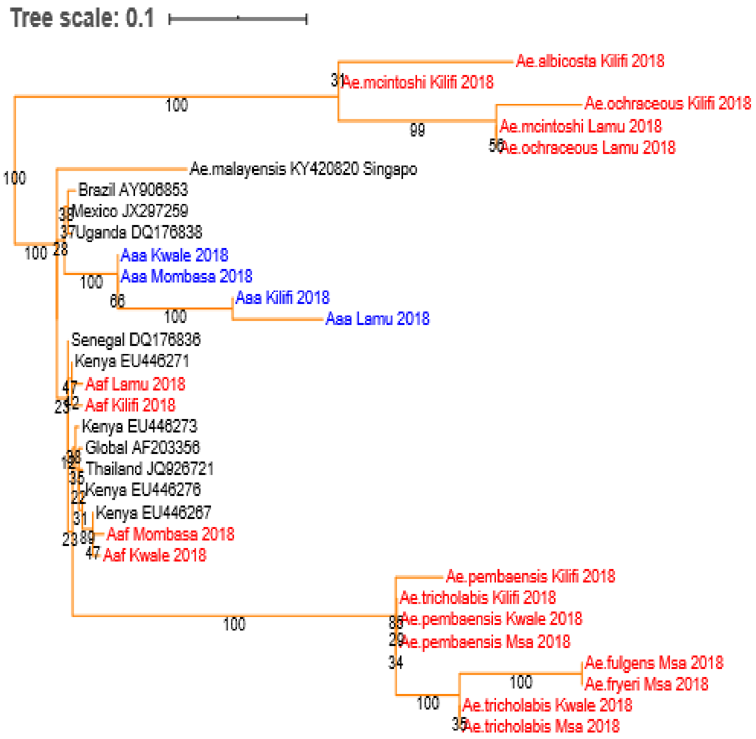


Figure 4.7: Maximum likelihood tree of the mtDNA NAD4 of *Aedes* mosquitoes sampled along the Coastline of Kenya and other parts of the world. Tree was constructed using clustal omega and RAxML version 8.1.20. Bootstrap values for 1000 replicates are indicated at the major branch points. New specie-haplotypes are highlighted in red. *Aedes aegypti aegypti* (highlighted in blue) was identified for the first time in this study along the Coastline of Kenya.

4.3 Phylogeny of Dengue and Chikungunya virus along the Coastline of Kenya

4.3.1 Proportions of Dengue virus along the Coastline of Kenya

An assay for the serotypes of Dengue virus was done in all the 1,861 pools of *Aedes* mosquitoes collected along the Coastline of Kenya during dry and wet seasons. Four serotypes of Dengue virus were identified: DENV-1, DENV-2, DENV-3 and DENV-4. Serotype DENV-4 was isolated in *Aedes* mosquitoes for the first time on the Coastline of Kenya and had the least vector infection rate in both seasons (Figure 4.8). 7.9% (147/1861 pools) of the *Aedes* mosquitoes were positive for Dengue virus with 59% of the cases detected during wet season. Although DENV-2 had the highest infection rate at an average of 54% in *Aedes* mosquitoes and in both seasons; the proportions of serotypes of DENV in *Aedes* mosquitoes along the Coastline of Kenya did not differ significantly with seasons (M=190, SD=159), t_3=0.1406, p>0.001.

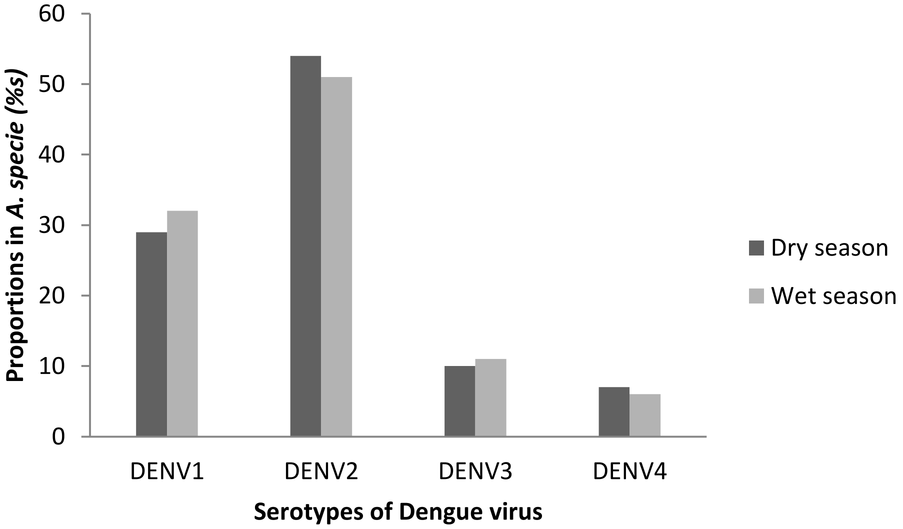


Figure 4.8: Proportions of serotypes of Dengue virus in *Aedes* species along the Coastline of Kenya during dry and wet seasons. DENV-4 was newly identified in this study and had the least infection rate in the *Aedes* mosquitoes.

4.3.2 Prevalence of Dengue virus along the Coastline of Kenya

Comparing prevalence's of Dengue virus infection in *Aedes* mosquitoes among Counties along the Kenyan Coastline: the prevalence of DENV-1 and DENV-2 in Mombasa was significantly higher than that of Lamu, *p*=0.025. The prevalence of DENV-3 was significantly higher in Kwale (*M*=3.4, *SED*=0.88), than in Lamu

(*M*=1.36, *SED*=0.9), *t*(12)=1.9, *p*=0.025. The prevalence of DENV-4 was significantly higher in Kwale (*M*=2.72, *SED*=1.0), than in Mombasa and Kilifi, *p*=0.025 as shown in Figure 4.9. However, the prevalence of Dengue virus in *Aedes* mosquitoes did not differ significantly with seasons, *p*>0.001.

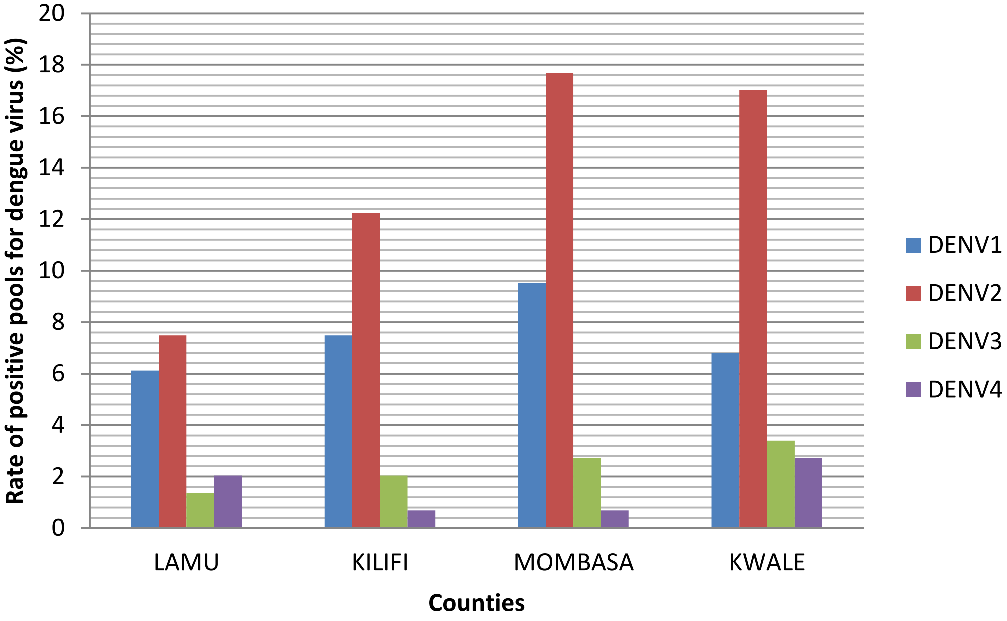


Figure 4.9: Prevalence of serotypes of Dengue virus along the Coastline of Kenya. DENV-2 had the highest prevalence across all counties.

4.3.3 Phylogeny of Dengue virus along the Coastline of Kenya

The 16 new haplotypes of Dengue virus identified along the Coastline were compared among themselves to estimate their phylogeny (Figure 4.10). Each serotype of Dengue virus (DENV-1, DENV-2, DENV-3 and DENV-4) formed an orthologous gene group. Dengue haplotypes from Kwale were closely related to those from Mombasa. Similarly, Dengue haplotypes from Kilifi were closely related to those from Lamu.

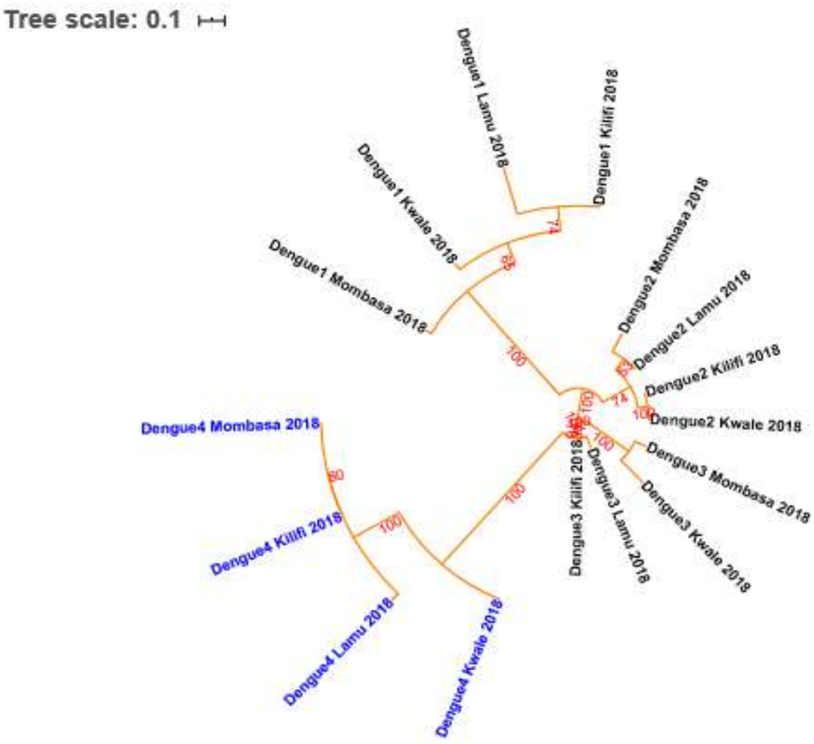


Figure 4.10: Phylogenetic relationships among serotypes of Dengue virus based on the nsp5 coding regions along the Coastline of Kenya. Tree based on the RAxML algorithm under the Tamura-Nei genetic distance model. Bootstrap values are marked on the branches. New serotype-haplotypes are highlighted in red. Serotype-haplotypes identified for the first time are highlighted in blue.

Comparing the new identified haplotypes to those identified earlier in Kenya and all over the world, all the serotypes of Dengue virus belonged to the same orthologous gene group with Yellow fever virus_MG757498 forming an out group (Figure 4.11). New haplotypes of DENV-3 were closely related to those previously isolated in Mombasa (KX812504; KX812511), Brazil (AY679147), India (AY770511) and China (KF954949). DENV-1 haplotypes were closely related to those previously isolated in Mombasa (KX812500) and India (JN903581).

Haplotypes of DENV-2 were closely related to those previously reported in Mombasa (KX812508), Pakistan (KF041236) and China (KT187558). The haplotypes of DENV-4 were closely related to those from Haiti (KP140942; JF262782).

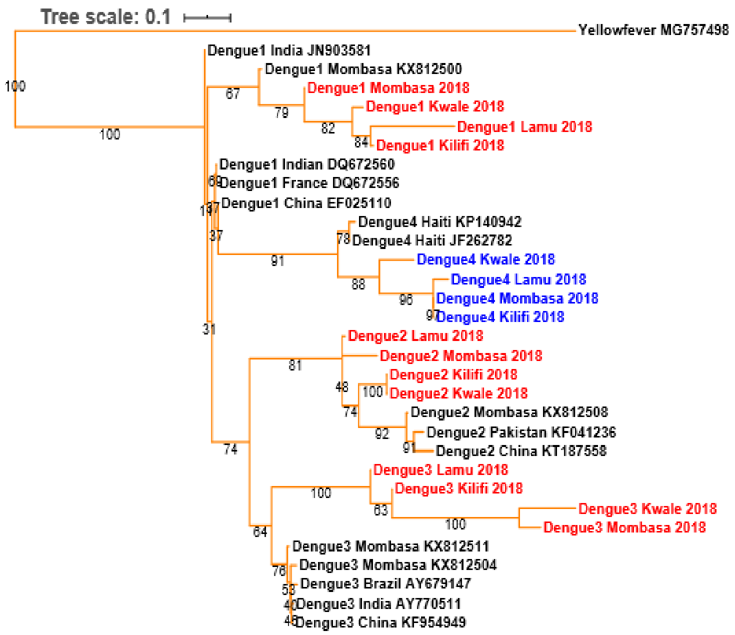


Figure 4.11: Maximum likelihood tree of serotypes of Dengue virus based on the nsp5 coding regions along the Coastline of Kenya. Sequences aligned using clustal omega and the phylogenetic tree constructed using RAxML version 8.1.20. Bootstrap values for 1000 replicates are indicated at the major branch points. New serotype-haplotypes are highlighted in red. Serotype-haplotypes identified for the first time are highlighted in blue.

4.3.4 Proportions of Chikungunya virus along the Coastline of Kenya

Molecular assay for genotypes of Chikungunya virus was done in all the 1,861 pools of *Aedes* mosquitoes sampled along the entire Coastline of Kenya during dry and wet seasons. Only the East Central and South African (ECSA) genotype of

Chikungunya virus was identified in this study. 2.1% (39/1861 pools) of the *Aedes* mosquitoes were positive for the virus with 58% of the positive cases identified during wet season (Figure 4.12). However, the proportions of this genotype in *Aedes* species along the Coastline of Kenya did not vary significantly with the seasons (M=7.25, SD=15), t_3=0.325, p>0.001.

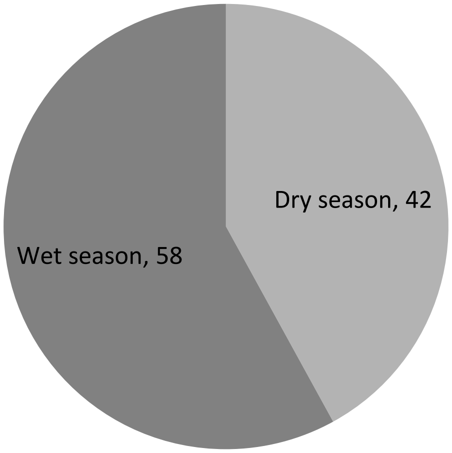


Figure 4.12: ECSA genotype of Chikungunya virus in *Aedes* species sampled along the Coastline of Kenya during dry and wet seasons.

4.3.5 Prevalence of Chikungunya virus along the Coastline of Kenya

Lamu County had the highest prevalence of CHIKV at 64% in *Aedes* mosquitoes as shown in Figure 4.13. However, comparing the prevalence's of CHIKV across the Counties, it was not significantly different (*M*=25, *SED*=26.18), *t*(57)=2.2, *p*=0.025.

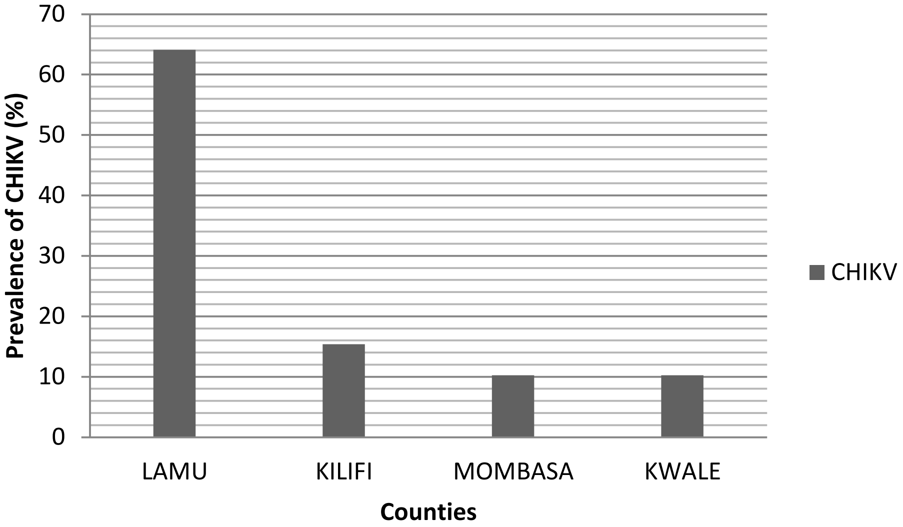


Figure 4.13: Prevalence of ECSA genotype of Chikungunya virus in *Aedes* mosquitoes sampled in counties along the Coastline of Kenya. Lamu County had the highest prevalence of Chikungunya virus at 64%.

4.3.6 Phylogeny of Chikungunya virus along the Coastline of Kenya

The Chikungunya haplotypes from Kwale were paraphyletic with respect to those from Mombasa, Kilifi and Lamu. Haplotypes from Mombasa, Kilifi and Lamu formed an orthologous gene group (Figure 4.14). The haplotypes form Kilifi were closely related to those from Lamu and they formed a monophyletic sister group.

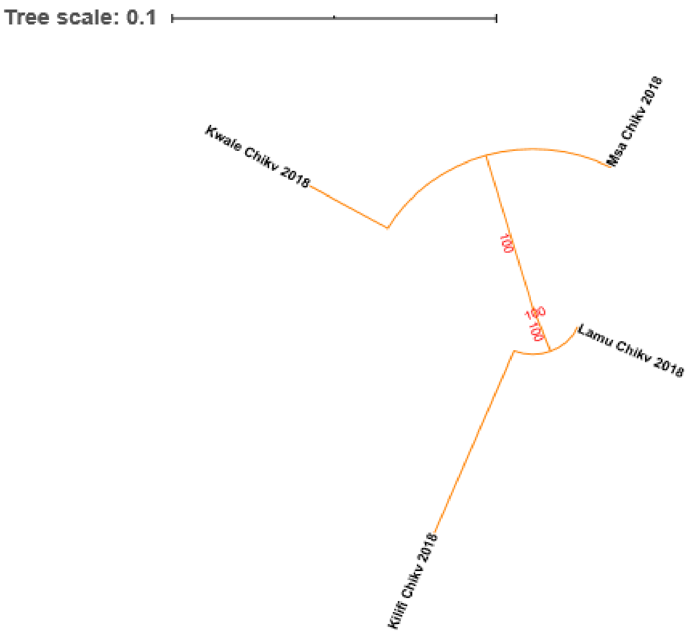


Figure 4.14: Phylogenetic relationships among Chikungunya virus haplotypes based on nucleotide sequence of the partial nsp1 gene along the Coastline of Kenya. Tree based on the RAxML algorithm under the Tamura-Nei genetic distance model. Bootstrap values for 1000 replicates are indicated at the major branch points. New genotype-haplotypes are highlighted in red.

Comparing the new haplotypes of Chikungunya to those previously reported in Kenya and all over the world, haplotypes from India (MH029068) formed an out group (Figure 4.15). The new haplotypes were closely related to those previously reported in Lamu (HQ456255), Mombasa (HQ456254), South Africa (HM045795), Tanzania (HM045811) and Congo (HM045809). Haplotypes from Asia were clustering together and haplotypes from West Africa were also clustering together.

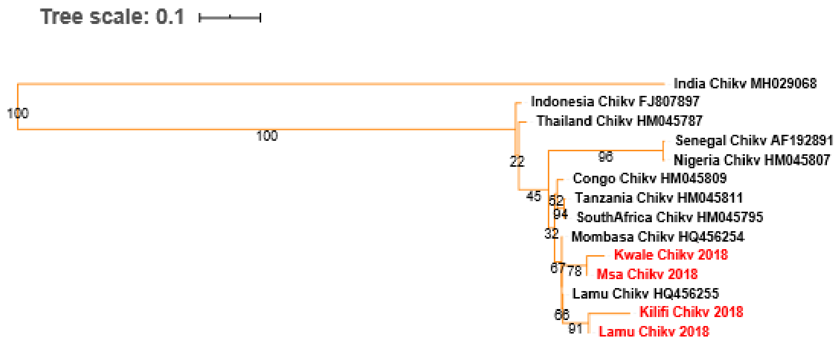


Figure 4.15: Maximum likelihood phylogenetic tree among Chikungunya virus haplotypes based on nucleotide sequence of the partial nsp1 gene along the Coastline of Kenya. Sequences aligned using clustal omega and the phylogenetic tree constructed using RAxML version 8.1.20. Bootstrap values for 1000 replicates are indicated at the major branch points. New genotype-haplotypes are highlighted in red.

4.3.7 Distribution of Dengue and Chikungunya viruses along the Coastline of Kenya

a) Dengue virus

The seasonal distribution of serotypes of Dengue virus along the Coastline of Kenya during dry and wet seasons was determined. All serotypes of Dengue virus were present on the entire Coastline during both seasons (Figures 4.16 and 4.17). However, DENV-3 was not isolated in *Aedes* mosquitoes collected from marine ecosystems. The distribution of the virus was uniform between and within dry and wet seasons. The newly identified DENV-4 was not present in *Aedes* mosquitoes sampled from Rabai and Mazeras sampling sites.

b) Chikungunya virus

The seasonal distribution of ECSA genotype of Chikungunya virus in *Aedes* species along the Coastline of Kenya was determined during dry and wet seasons.

The distribution of the virus was uniform between and within dry and wet seasons. However, Chikungunya virus was highly concentrated on the northern parts of the Coastline: sites of Malindi and Lamu as shown in Figures 4.16 and 4.17.

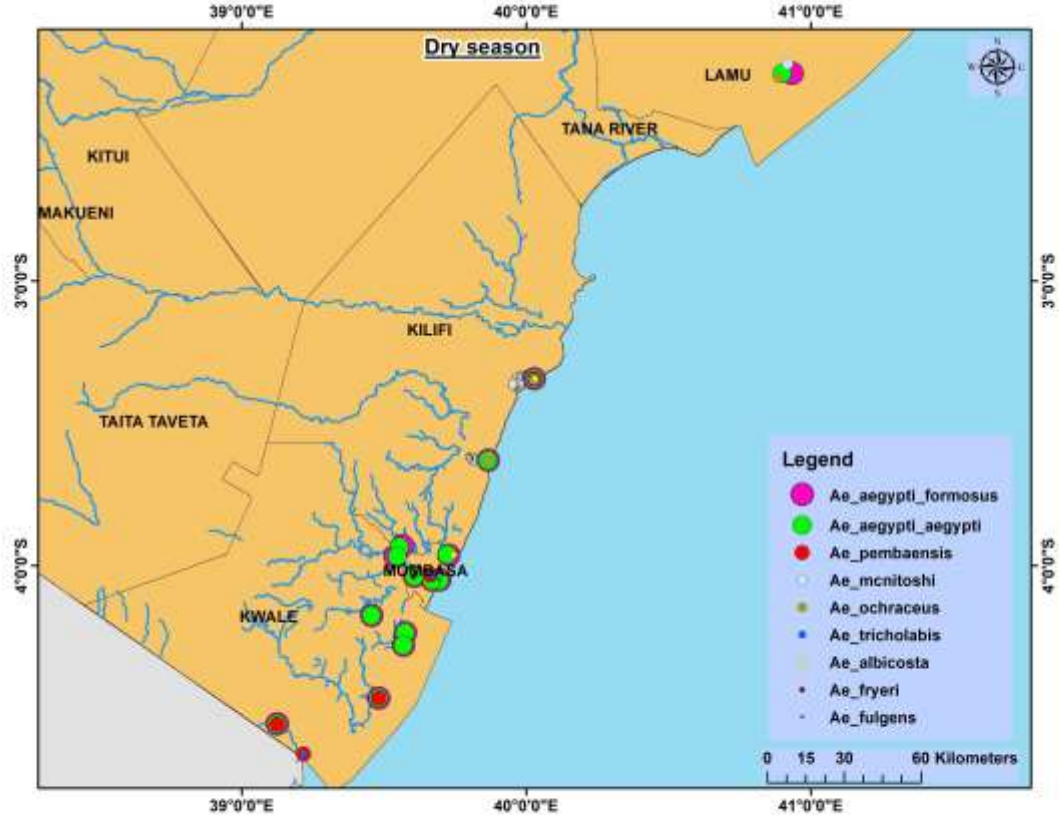


Figure 4.16: Distribution of serotypes of Dengue virus along the Coastline during dry season. The size of the shape is directly proportional to the rate of infection of *Aedes* species by the serotype. Serotype DENV-1 and DENV-2 were distributed along the entire Coastline.

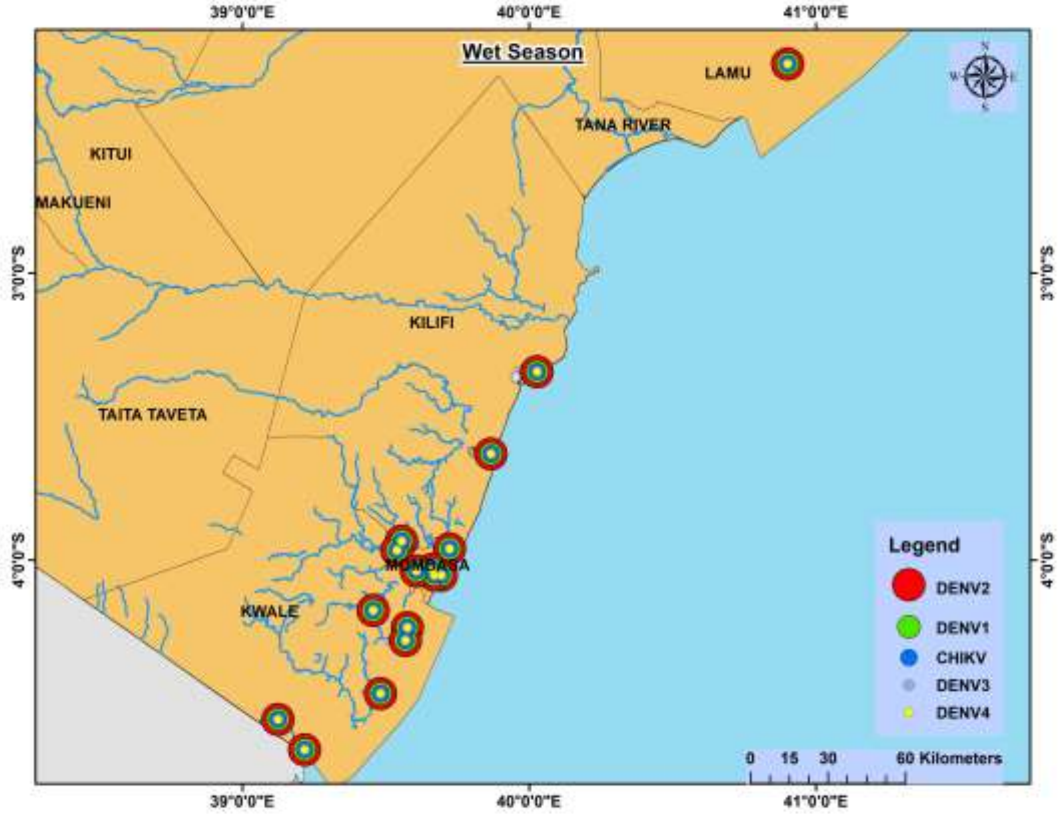


Figure 4.17: Distribution of serotypes of Dengue virus along the Coastline during wet season. The size of the shape is directly proportional to the rate of infection of *Aedes* species by the serotype. Serotype DENV-1 and DENV-2 were distributed along the entire Coastline.

4.4 Co-infections of Dengue and Chikungunya viruses in *Aedes* mosquitoes along the Coastline of Kenya

Screening for infections of Dengue and Chikungunya viruses was done in both male and female *Aedes* mosquitoes collected along the Coastline of Kenya during dry and wet seasons.

4.4.1 Dengue and Chikungunya virus in males and females of *Aedes* mosquitoes

Proportions of males and female *Aedes* mosquitoes infected with either Dengue or Chikungunya or with both viruses are shown in Figure 4.18. Although infection rates were higher in the females, analysis of variance showed that sex orientation does not have influence on infection by the viruses, *F*(1,5)=6.61,*p*=0.05

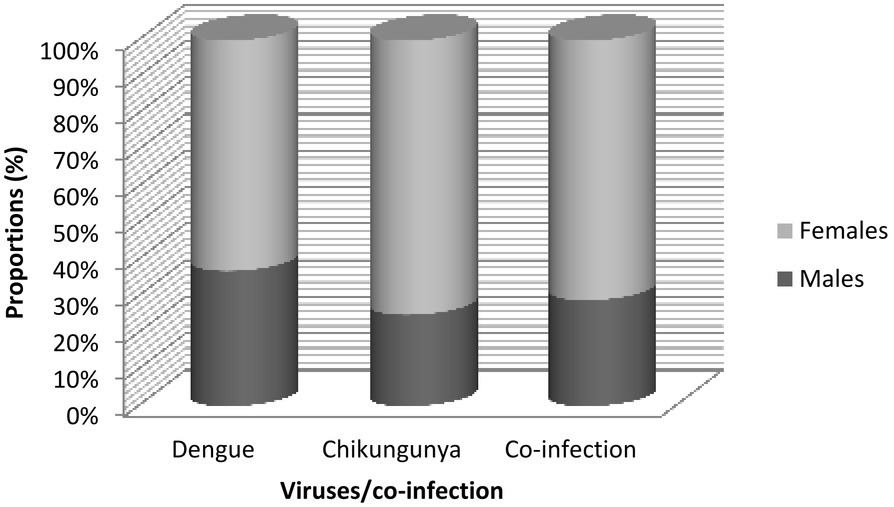


Figure 4.18: Proportions of Dengue, Chikungunya viruses and their co-infection in male and female *Aedes* mosquitoes.

4.4.2 Proportions of Dengue and Chikungunya viruses along the Coastline of Kenya.

Pools of *Aedes* mosquito were analysed for proportions positive for virus infections. DENV-2 had the highest vector infection rate at 43% (80/186 pools), while DENV-1 at 24%, DENV-3 at 7%, DENV-4 at 5% and CHIKV at 21% (Figure 4.19). The proportion of DENV-2 in *Aedes* mosquitoes was significantly higher (M=20, SED=15.33), t(12)=33.40, p=0.025.

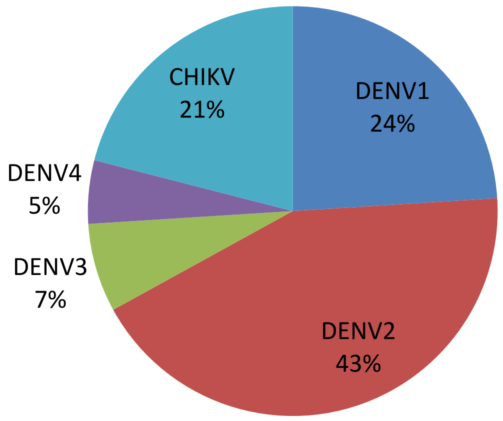


Figure 4.19: Proportions of Dengue and Chikungunya viruses along the Coastline of Kenya.

4.4.3 Co-infections of Dengue and Chikungunya viruses in *Aedes* species along the Coastline of Kenya

6% (104 pools) of *Aedes* mosquitoes had coinfection of Dengue and Chikungunya viruses. The results shown in Figure 4.20 indicate *Aedes aegypti formosus* and *Aedes aegypti aegypti* were positive for all serotypes of Dengue and Chikungunya viruses with DENV-2 isolated in all *Aedes* species. *Aedes tricholabis* and *Aedes fulgens* were negative for Chikungunya virus.

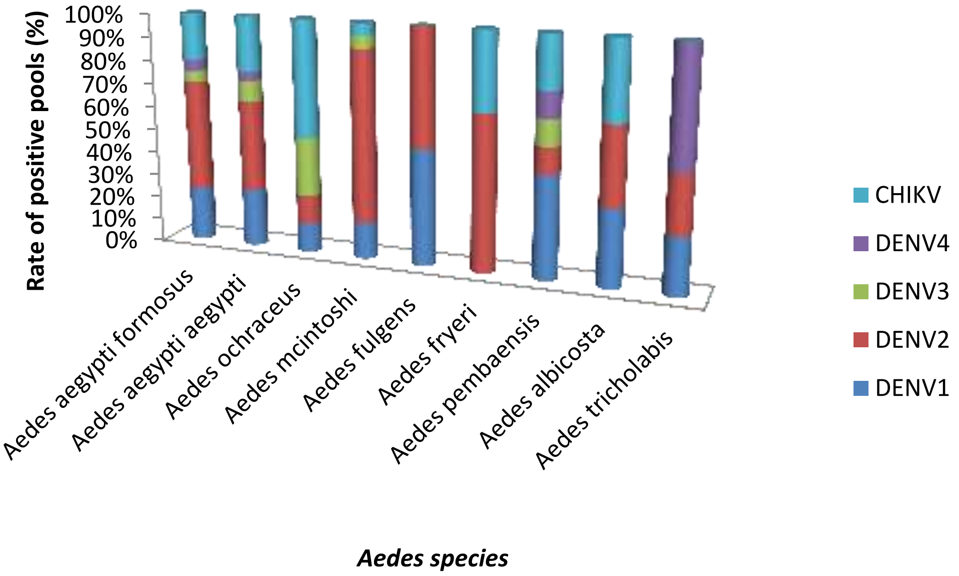


Figure 4.20: Dengue and Chikungunya viruses in *Aedes* species along the Coastline of Kenya. DENV-2 was isolated in all *Aedes* species.

Aedes aegypti formosus had the highest pools of co-infections at 43% with infections of DENV-1, DENV-2, DENV-3, DENV-4 and CHIKV as shown in Figure 4.21. *Aedes aegypti formosus* had a significant rate of pools with co-infections (*M*=11.2, *SED*=14.10), *t*(27)=28.93, *p*=0.025, except when compared with *Aedes aegypti aegypti*.

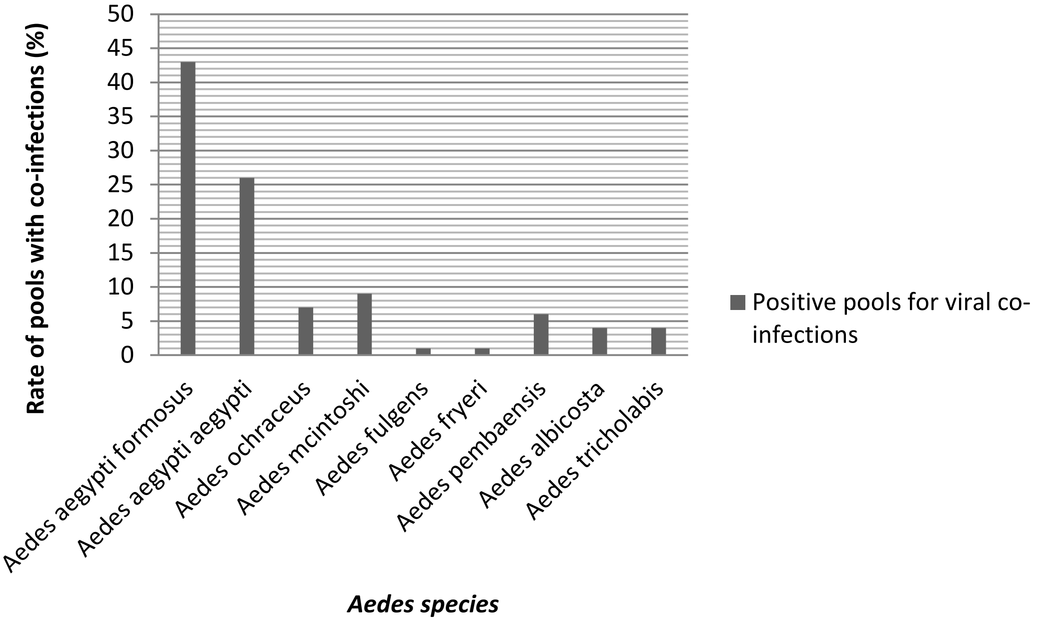


Figure 4.21: Co-infection of Dengue and Chikungunya viruses in *Aedes* mosquitoes along the Coastline of Kenya.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

This was the first study to document the epidemiology of Dengue and Chikungunya viruses in various species of *Aedes* mosquitoes along the entire Coastline of Kenya during dry and wet seasons. Similarly, this was the pioneer study to identify serotypes of Dengue virus and genotypes of Chikungunya virus in *Aedes* mosquitoes and analyse for their evolutionary relationships. Previous studies at the coastal region of Kenya on vectors of arboviruses were in specific areas for limited periods of time. These specific areas include Rabai, Watamu and Kwale and the studies were conducted by (Edwards, 1941; Sang *et al.,* 2010; Moore *et al.,* 2013; Ndenga *et al.,* 2017; Ngugi *et al.,* 2017; Sang *et al.,* 2017; Iwashita *et al.,* 2018) among others.

5.1.1 Spatial and temporal distribution of *Aedes* mosquitoes along the Coastline of Kenya

Composition of *Aedes* mosquitoes along the Coastline of Kenya

In the present study, *Aedes aegypti aegypti* was identified for the first time along the Coastline of Kenya. Previous studies identified this *Aedes* sub-species in Shauri Moyo village in Rabai (Trpis and Hausermann, 1986), which is inland from the coastal region of Kenya. *Aedes aegypti aegypti* is a sub species of *Aedes aegypti* s.l and was previously reported in East Africa, South America, Caribbean and West Africa (Tabachnick, *et al.,* 1985). This identification of *Aedes aegypti*

aegypti could be attributed to the fact that sampling and analysis of *Aedes* mosquitoes was for the entire Coastline for a continuous period of time (two years including dry and wet seasons). In addition, there was a possibility of gradual expansion of the geographical niche of these vectors or possible mutations within members of *Aedes aegypti* s.l resulting into distribution of this sub-species on the Coastline as hypothesised earlier (Kraemer *et al.,* 2015).

Other *Aedes* mosquitoes identified in this study along the Coastline of Kenya include: *Aedes aegypti formosus, Aedes pembaensis, Aedes ochraceus, Aedes mcnitosh, Aedes tricholabis, Aedes fryeri, Aedes fulgens* and *Aedes albicosta*. Identification of these *Aedes* species agreed with previous reports in selected parts of coastal Kenya (Edwards, 1941; Ngugi *et al.,* 2017; Ndenga *et al.,* 2017; Iwashita *et al.,* 2018; Moore *et al.,* 2013 and Sang *et al.,* 2010). The finding that the composition of *Aedes* species was not significantly different between dry and wet seasons implied that seasons had no impact on the composition of *Aedes* species present in the region.

Proportions of *Aedes* mosquitoes along the Coastline of Kenya

Although more *Aedes* mosquitoes were collected during the wet season, their proportions were not significantly different along the Coastline in both seasons; this indicated seasons had no impact on the proportions of *Aedes* mosquitoes. Similar findings were reported earlier for *Aedes bromeliae* (Agha *et al.,* 2017). This could be due to abundance and sustenance of breeding sites during both seasons. Such breeding sites could include holes on tree trunks and water storage containers as earlier reported (Lutomiah *et al.,* 2016), and therefore there is potential of transmission of the viruses throughout the year.

Proportion of Sex of *Aedes* mosquitoes along the Coastline of Kenya

The observation that the proportion of males and female *Aedes* mosquitoes was not significantly different indicated that season did not influence sex of *Aedes* mosquitoes in this region. Similar findings were reported in *Aedes aegypti* collected in Argentina (Grech *et al.,* 2010). This could be explained by the fact that sampling was both outdoor and indoor as earlier reported (Chadee, 2012; Harrington *et al.,* 2014; Agha *et al.,* 2017). Outdoor was in bushes surrounding homesteads where most male *Aedes* mosquitoes feed on fruit juices and flower nectars while indoor was in houses where most female *Aedes* mosquitoes have been reported to inhabit as they look for a blood meal which is necessary for viability of their eggs (Day, 2016). *Aedes pembaensis* and *Aedes tricholabis* exclusively inhabited the mangrove ecosystems and had their female to male proportion almost constant during both seasons. This could be due to the availability of breeding sites (crab burrows) throughout the year in the mangrove ecosystems as previously reported (Lardeux *et al.*, 2002; Sang *et al.*, 2010).

Prevalence of *Aedes* mosquitoes along the Coastline of Kenya

The finding that prevalence of *Aedes aegypti formosus* was significantly higher along the Coastline agreed with findings by (Agha *et al.,* 2017), although they analysed mosquitoes from Kilifi site only in the coastal region of Kenya. Perhaps this could be due to the intensive outdoor sampling carried out in this study in relation to the feeding and resting behaviour of these two *Aedes aegypti* sub-species. *Aedes aegypti formosus* is known to be an outdoor mosquito primarily feeding on wild animals (exophagic and zoophilic); while *Aedes aegypti aegypti* as an indoor sub-species preferring to feed on human blood (endophagic and anthropophilic) (Mattingly, 1967).

Distribution of *Aedes* mosquitoes along the Coastline of Kenya

The fact that distribution of *Aedes* species along the Coastline of Kenya was similar during dry and wet seasons implied that seasons did not affect how these mosquitoes were distributed in this region. The finding that members of *Aedes aegypti s.l* were absent in marine ecosystems could indicate marine ecosystems may not be the preferred breeding grounds for members of *Aedes aegypti s.l.* However, studies should be done to assess any influence of marine ecosystems on the bionomics of *Aedes aegypti s.l* mosquitoes, for example, their breeding behaviour in relation to the marine ecosystems.

Identification of *Aedes ocharaceous* and *Aedes mcintoshi* in Lamu and Kilifi Counties confirmed earlier reports that these vectors were present in the northern parts of the Coastline in Tana-River, Isiolo and Garissa (Ochieng *et al.*, 2013; La beaud *et al.*, 2015; Sang *et al.*, 2017). This observation maybe explained by two possible hypotheses. First, it may imply *Aedes ochraceus* and *Aedes mcnitosh* are expanding their geographical distribution southwards and this could be made possible by the increased human traffic between Garissa and areas of Malindi, Kilifi and Mombasa for religion, economic and social activities. Secondly, the isolation of these species of *Aedes* mosquitoes may be attributed to the sampling of more sites which were not sampled in the previous studies on the Coastline of Kenya.

In this study, *Aedes fryeri* and *Aedes fulgens* were present only in the coastal sites of Mombasa Island and Port reiz, while *Aedes albicosta* were present only in Malindi sampling sites. This finding agreed with earlier reports (Edwards, 1941) and may indicate members of *Aedes fryeri* and *Aedes fulgens* have not expanded their geographical distribution to include other areas of the coastal region as sampling was done on the entire Coastline. Identification of *Aedes albicosta* in Malindi may be due to expansion of sampling region or the vector is expanding its geographical distribution northwards as it was earlier reported in Mombasa (Edwards, (1941). *Aedes pembaensis* and *Aedes tricholabis* were the only *Aedes* species identified in marine ecosystems. In addition, they were only found in marine ecosystems. This finding for *Aedes pembaensis* agreed with earlier reports (Sang *et al.,* 2010), although this mosquito was identified alongside other *Aedes* species. Therefore, this becomes the first study to document identification of only members of *Aedes pembaensis* and *Aedes tricholabis* in marine ecosystems along the Coastline of Kenya. This could be due to migration of the other *Aedes* species due to breeding challenges as members of *Aedes pembaensis* and *Aedes tricholabis* are able to utilise holes made by ocean crabs for breeding. Identification of *Aedes tricholabis* in Kilifi confirmed earlier findings (Agha *et al.,* 2017). However, *Aedes tricholabis* was also present in the Counties of Kwale and Mombasa implying it could be migrating southwards along the Coastline of Kenya.

It is evident that most *Aedes* species were expanding their geographical distribution and this phenomenon could be possible influenced by factors like urbanization, globalization, presence of airports and airstrips, automobiles which facilitate movements of people and goods thus carrying mosquitoes along with them as earlier reported Harrington *et al.,* (2014 and Konongoi *et al.,* (2016). This frequently occurs along the coastline with movement of people and goods among the towns of Garissa, Lamu, Malindi, Kilifi, Mombasa and Kwale.

5.1.2 Phylogeny of *Aedes* mosquitoes along the Coastline of Kenya

The fact that *Aedes aegypti formosus* and *Aedes aegypti aegypti* formed an orthologous gene group indicated a close phylogenetic relationship between them. This finding could mean a common ancestral origin and a close evolutionary path between *Aedes aegypti formosus* and *Aedes aegypti aegypti* as earlier documented (Harrison and Langdale, 2006; Paupy, *et al.,* 2012). The formation of a sister group by *Aedes pembaensis* and *Aedes tricholabis* implies a close evolutionary relationship between them. This close evolutionary relationship could be the basis for a common breeding behaviour in marine ecosystems. The close evolutionary relationship between *Aedes fryeri* and *Aedes fulgens* might be attributed to the fact that these mosquitoes are found in similar geographical ecosystems within Mombasa Island; therefore a possibility of sharing some geographical adaptive traits. A similar inference could be drawn for *Aedes mcnitosh* and *Aedes ochraceus* for their preference to breed in flood waters (Ochieng *et al.,* 2013). Sharing of orthologous gene group by *Aedes* mosquitoes in the out group implies a close evolutionary relationship among them. It is observed that organisms sharing a group of orthologous genes have a close evolutionary relationship and this could be due to sharing of common ancestral origin by members of these species (Paupy, *et al.,* 2012).

In addition to the earlier haplotypes of *Aedes aegypti* s.l identified (Moore *et al.,* 2013), eight (8) new haplotypes of *Aedes aegypti* s.l were documented for the first time in this study. The isolation of new haplotypes could be due to sampling of the entire Coastline for consecutive two years. In addition, evolution of these *Aedes* species may have occurred with time and space due to environmental selection pressure, for example, challenges by insecticides, decrease in breeding sites and climate change especially increase in temperature (Kraemer *et al.,* 2015). The finding that all the 8 new haplotypes of *Aedes aegypti s.l* were in the upper clade of the phylogenetic tree together with those of *Aedes pembaensis*, *Aedes tricholabis*, *Aedes fulgens* and *Aedes fryeri* indicated a close relationship among them.

The absence of the new haplotypes of *Aedes aegypti* s.l in the basal clade of the in group agrees with earlier findings that most *Aedes* mosquitoes from East African region fall on the upper clade (Moore *et al.,* 2013). The identification of new haplotypes call for studies to analyse any dynamics in fitness traits like fecundity, insecticide resistance, longevity, preferred breeding sites among others. *Aedes aegypti formosus* haplotypes were closely related to those from Saudi Arabia and Thailand and this may be attributed to the fact that there exist a lot of movement of goods and people between Kenya and the Asian continent thus introducing these mosquitoes between these regions. On the other hand, *Aedes aegypti aegypti* haplotypes were observed to be closely related to those from Uganda, Mexico and Brazil and this may still be due to the improved movements and goods between these countries as earlier described (Harrington *et al.,* 2014).

5.1.3 Phylogeny of Dengue and Chikungunya viruses

Serotypes of Dengue virus

This study reports for the first time the presence of serotype DENV-4 in *Aedes* mosquitoes along the Coastline of Kenya. This finding agreed with earlier serological assays in Mombasa by Sutherland *et al.,* (2011). However, this finding was contrary to assay reports by Konongoi *et al.,* (2016) in which only three Dengue serotypes (DENV1-3) were identified. This may be due to the expansive geographical area of sampling and the fact that entomological assays were done contrary to the earlier studies. The result that serotype DENV-3 was not identified in *Aedes* mosquitoes sampled from marine ecosystems may be due to inability of the virus to carry out its physiological functions in *Aedes* mosquitoes inhabiting marine conditions; or due the presence of only *Aedes pembaensis* and *Aedes tricholabis* in this ecosystems which might not be their preferred invertebrate hosts.

Serotype DENV-5 was not identified in *Aedes* mosquitoes in this study, and this agreed to reports from earlier serological assays for patients presenting with fever at various health facilities in Mombasa (Sutherland *et al.,* 2011; Konongoi *et al.,* 2016) but contrasts findings by (Normile, 2013) that serotype DENV-5 was present in non-human primates (macaque monkeys) and is transmitted by *Aedes* mosquitoes in Malaysia. However, the absence of serotype DENV-5 in *Aedes* mosquitoes along the Coastline of Kenya could be due to the fact that this serotype was only recently identified in Malaysia far from the coastal strip of Kenya. Therefore, future studies should also assay for serotype DENV-5 in vectors, non-human primates and humans as increased human movement between Kenya and Malaysia might result into the introduction of the serotype in Kenya.

Composition and prevalence of serotypes of Dengue virus in *Aedes* mosquitoes

The fact that the composition and prevalence of Dengue virus was not significantly different between dry and wet seasons implied the seasons don’t influence their presence in the vectors. However, the abundance of DENV-2 in both seasons indicated this virus was the commonest along the Coastline of Kenya. This result agreed with earlier findings (Sutherland *et al.,* 2011; Ochieng *et al.,* 2015; Ngoi *et al.,* 2016); but contradicted observations by Konongoi *et al.,* (2016) in which DENV-1 was reported to have a higher prevalence at 44% compared to that of DENV-2 at 39%. The high prevalence of DENV-2 in the mosquito population during dry season explains the occurrence of most Dengue outbreaks following rain season along the coast of Kenya.

Serotype DENV-4 was the least circulating serotype of Dengue virus in *Aedes* species along the Coastline of Kenya and this agreed with findings by (Sutherland *et al.*, 2011). The lower levels of serotype DENV-4 along the Coastline of Kenya could be due to its recent introduction in the region. However, its entomological and human infection levels may increase with time if appropriate control measures are not instituted as it is currently present in all Counties along the Coastline. Despite different infection levels of these serotypes of Dengue virus in *Aedes* species, the infection rates did not vary significantly along the Coastline and with seasons. This meant all serotypes of Dengue virus were in circulation in the *Aedes* mosquito populations and human infection could occur in similar proportions upon exposure to infected *Aedes* mosquito bites.

The fact that the prevalence of Dengue viruses in *Aedes* mosquitoes did not differ significantly with seasons implied seasons did not influence susceptibility of the mosquitoes to the virus. However, their rates of infection increased during wet season which could result to Dengue fever outbreak. This was similar for Chikungunya virus. The increased infection rates in *Aedes* mosquitoes could be attributed to increased number of *Aedes* mosquitoes due to readily available breeding grounds and up scaled human contacts during wet seasons. Increased human contacts could result from increased agricultural and economic activities like bush clearing, farming, fetching of firewood and grazing activities/pastoralism. This could increase rates of human infection as *Aedes* mosquitoes rest in these bushes during the day and people are at risk of being infected with the virus upon a bite by an infected female *Aedes* mosquito. Similar risk factors were previously reported (Grossi-Soyster, *et al.*, 2017). The fact that Mombasa County had the highest prevalence of Dengue infections at 30.6% in *Aedes* mosquitoes could explain the frequent outbreaks of Dengue fever in this county (www.health.go.ke).

Phylogeny of Dengue virus along the Coastline of Kenya

The identification of 16 more new haplotypes {in addition to previously 12, (Konongoi *et al.,* 2016)} of Dengue virus could have been due to expansion of the study area and duration of the study. In addition, the presence of additional *Aedes* species on the Coastline and selection pressure such as ecological dynamism may have influenced evolutionary processes in the virus. Similar hypothesis was put forward in phylogenetic analysis of Chikungunya virus (Volk *et al.,* 2010).

The finding that all haplotypes of Dengue virus from Kwale and Mombasa; and from Kilifi and Lamu were forming distinct sister clades indicated a close phylogenetic relationship between them. This could be attributed to close proximity between Kwale and Mombasa, and between Kilifi and Lamu with improved traffic between these respective Counties with travellers carrying strains of these viruses in their bodies or in *Aedes* mosquitoes in automobiles as earlier hypothesised in China (Wang *et al.,* 2015). The formation of three orthologous gene groups by the serotypes of Dengue virus confirms previous reports (Konongoi *et al.,* 2016) although Dengue- 4 was not identified in their findings. The presence of DENV-4 in Kenya could be due to the increased number of people travelling between Haiti and Kenya. The fact that all serotypes shared a common node implies they had a common ancestry therefore a higher possibility of similarity in their evolutionary path as earlier indicated (Anez Germa'n, *et al.,* 2012).

Genotypes and prevalence of Chikungunya virus in *Aedes* mosquitoes

Analysis of genotypes of Chikungunya virus in *Aedes* mosquitoes sampled along the Coastline of Kenya identified only one genotype; the East Central and South African (ECSA). This finding was in line with reports from recent studies (Sergon *et al.,* 2008; Njenga *et al.,* 2008). Absence of the west African and Asian genotypes of Chikungunya virus from all these studies could be attributed to the ability of the genotypes to physiologically adapt and manifest themselves in the East, Central and South African regions due to their genotypes (as their names suggest). The finding that the prevalence of the virus was not significantly different between wet and dry seasons implied season did not influence its prevalence in *Aedes* mosquitoes. This may be due to the availability of indoor and outdoor breeding sites in both seasons as reported earlier (Ngugi *et al.,* 2017). However, the high level of prevalence of the virus in the mosquito population during wet season explains the occurrence of Chikungunya outbreaks during rain seasons along the coastline of Kenya.

Prevalence of Chikungunya virus in Counties along the coastline of Kenya

The finding that the prevalence of Chikungunya virus was not significantly different across all Counties implied all Counties along the Coastline of Kenya were at an equal risk of infections upon being bitten by *Aedes* mosquitoes. In terms of its distribution, this genotype was present in *Aedes* species along the entire Coastline of Kenya. However, the virus was common in *Aedes* mosquitoes from the northern regions of the Coastline particularly in Lamu. This could be due to the existence of this virus for a long period of time in this region, as the first outbreak was reported Lamu County in 2004 (Njenga *et al.,* 2008). Identification of Chikungunya virus in Mombasa confirmed serological findings by (La Beaud *et al.,* 2015) but was in contrast with reports by (Ngoi *et al.*, 2016) who reported 0% infection. These contrasting results by the previous studies may be due to analysis of serum samples in single sites within a limited period of time in the coastal region of Kenya.

Phylogeny of Chikungunya virus along the Coastline of Kenya

The fact that the haplotypes of Chikungunya virus isolated in *Aedes* mosquitoes along the Coastline shared an orthologous gene group means they originated from a common ancestor. The observation that haplotypes from Lamu formed a cluster with those from Kilifi; and those from Mombasa with those from Kwale indicated a close phylogenetic relationship between them and this may be due to proximity of these Counties and frequency of people travelling across borders of these regions. The finding that haplotypes of Chikungunya virus identified previously and in this study along the coastal strip were closely related to those from South Africa, Tanzania and Congo suggests a close evolutionary relationship among these viruses which could be made possible due to historical relationships since the 17th and 18th centuries between African countries. The observation that the west Africa Chikungunya genotypes (WAG) were paraphyletic to Sindbis and Onyong-nyong virus could suggest a close evolutionary relationship among these viruses.

5.1.4 Co-infections of Dengue and Chikungunya viruses in *Aedes* mosquitoes

In this study, male *Aedes* mosquitoes were found to be infected with Dengue and Chikungunya viruses although sex did not influence the status of infection. This finding agreed with earlier assays (Lutomiah *et al.*, 2016). The infection of males of *Aedes* mosquitoes clearly demonstrated the ability of male mosquitoes to play a role in transmission of these viruses in the environment. Therefore, control measures for arboviral diseases should target both males and females.

Absence of serotype DENV-4 in *Aedes macnitosh* from Lamu, Malindi and Kilifi may suggest that *Aedes macnitosh* were not suitable hosts for DENV-4. Similar observations were made for *Aedes tricholabis* with Chikungunya virus despite the virus being detected in *Aedes pembaensis* in the same sites of Watamu, Tudor and Vanga. This was also observed for *Aedes fryeri* with DENV-1, DENV-3 and DENV-4 in Port Reiz. This calls for investigation to establish the vector-host-pathogen relationships in order to understand their mode of interaction (either biochemical or physiological). Such knowledge will be paramount in development of arboviral control strategies, for example, transmission blockage vaccines.

The isolation of new haplotypes of Dengue and Chikungunya viruses along the Coastline of Kenya clearly demonstrated genetic dynamisms in these viruses. Such genetic changes should be investigated in respect to traits like pathogenicity, virulence, and host and vector preference. The findings that serotypes of Dengue and the genotype of Chikungunya viruses occurred in similar geographical regions during dry and wet seasons along the Coastline of Kenya suggested these viruses could overlap as previously reported (Kosasih *et al.,* 2016; Le Coupanec *et al.*, 2017). The observed selective infection of *Aedes* species by serotypes of Dengue and genotypes of Chikungunya viruses in the same regions may suggest preferential association among viruses, vectors and hosts.

The finding that all *Aedes* species had concomitant infections of Dengue and Chikungunya viruses implied each species can transmit these viruses to their hosts along the Coastline as reported earlier (Le Coupanec *et al.*, 2017). The fact that members of *Aedes aegypti* s.l had multiple infections of all serotypes of Dengue and Chikungunya virus could mean they have higher vector competence thus efficient vectors of these viruses along the Coastline of Kenya as previously alluded (Mattingly, 1967; Tabachnick, *et al.*, 1985; Rückert *et al.*, 2017).

It is worth noting that arboviruses could share a geographical area or overlap (Konongoi *et al.,* 2018). Chikungunya and Zika viruses could occur and co-circulate in same geographical zones, while Dengue and Chikungunya could overlap with Dengue endemic areas (Göertz *et al.,* 2017). Individuals living in these areas could get multiple infections from the same infected mosquito or different mosquitoes (Chaves, *et al.*, 2018). Co-infection could be symbiotic where the transmission of one virus is aided by the presence of the other, as in the case of co-infection of Dengue and Zika virus (Chaves, *et al.*, 2018). Cases of arboviral co-infections in *Aede*s mosquitoes have been reported in areas with reports of arboviral co-infections in humans emphasising need to analyse co-infections in the vector mosquitoes. Such knowledge could be instrumental in development of effective control programs by the Ministry of health for arboviral infections especially in hot spots and endemic areas.

5.2. Conclusions

i. The spatial and temporal distribution and proportions of *Aedes* species was not affected by seasons along the Coastline of Kenya.

ii. *Aedes* mosquitoes along the Coastline of Kenya belonged to eight (8) species and fall in the derived/ upper clade of the phylogenetic tree and closely related to isolates from Uganda, Mexico and Brazil.

iii. There were four Dengue serotypes (DENV1-4) and one genotype of Chikungunya virus (ECSA). DENV-1 and DENV-2 isolates were closely related to those previously isolated in Mombasa. DENV-3 isolates were closely related to those previously identified in Mombasa, Brazil, India and China. Chikungunya isolates were closely related to those previously identified in Lamu, Mombasa, South Africa, Tanzania and Congo.

iv. There were co-infections of Dengue and Chikungunya viruses in *Aedes* species found along the Coastline of Kenya.

5.3 Recommendations

1. The Ministry of Health: I recommend for continuous surveillance of vector epidemiology and assays for arboviruses to give an updated picture on their epidemiology and infection status to estimate the risk of arboviral outbreaks in the region.
2. The Ministry of Health: I recommend for public health education to reduce on the breeding sites for the *Aedes* mosquitoes. These include proper disposal of used containers and tyres, clearing of bushes and the use of insect repellents.
3. The Ministry of Health: Control measures for arboviral diseases should target both males and females as both sexes carry the viruses in the environment.
4. The ministry of Health: Automobiles, sea vessels, aircrafts and aeroplanes should be treated/sprayed where possible with insecticides before travelling to their destinations to avoid introduction of mosquitoes in their counties/countries of destination
5. Medical research: I recommend for serological assays for DENV-4 to find out if the virus is also circulating in the human population.
6. Medical research: I recommend for entomological and serological assays for DENV-5 in mosquito, non-human primates and human population to find out if the virus is in Kenya.
7. Agricultural and social practices: Farming and firewood collection activities should be done between 1000Hrs to 1500Hrs to avoid being bitten by *Aedes* mosquitoes which might be infected by the viruses. These mosquitoes are resting in bushes between these periods of time. Or they

can apply insecticide repellents like DEET whenever carrying out outdoors activities in such environments.

5.4 Study limitations

In this study, the main limitation was inability to sequence all the cDNA locally due to monetary resources; thus some samples were exported to Bernhard Nocht Institute for Tropical Medicine in Germany. However, the results were reported back and analysis was done successfully. In addition, analysis for indoor and outdoor samples was not done separately.

5.5 Further Research

i. Studies on how marine ecologies influence bionomics of *Aedes aegypti* s.l and DENV-3.

ii. Studies on assays for concomitant infections of arboviruses in individual *Aedes* species.

iii. Studies on how genetic changes in *Aedes* species, Dengue and Chikungunya viruses influence vector fitness traits and pathological related traits.

REFERENCES

Abu-Asab, M., Chaouchi, M. and Amri, H. (2008). Evolutionary medicine: A meaningful connection between omics, disease, and treatment. *Proteomics - Clinical Applications*. https://doi.org/10.1002/prca.200780047.

Agha, S. B., Chepkorir, E., Mulwa, F., Tigoi, C., Arum, S., Guarido, M. M., Ambala, P., Chelangat, B., Lutomiah, J., Tchouassi, D. P., Turrel, M. J. and Sang, R. (2017). Vector competence of populations of *Aedes aegypti* from three distinct cities in Kenya for Chikungunya virus. *Neglected Tropical Diseases* ***11*****(8):** e0005860.

Ali, K. H. L., El-Badry, A. A., Ali, M. A., El-Sayed W. S. M. and El-Bashbishy, H. E. (2016). Phylogenetic analysis of *Aedes aegypti* based on mitochondrial ND4 gene sequences in Almadinah, Saudi Arabia. *Iranian Journal of Biotechnology* ***14*****(12):** 58-62.

Bhatt, S., Gething, P. W., Brady, O. J., Messina, J. P., Farlow, A. W., Moyes, C. L., Drake, J. M., Brownstein, J. S., Hoen, A. G., Sankoh, O., Myers, M. F and Hay, S. I. (2013). The global distribution and burden of Dengue. *Nature* **498(7446):** 504-507.

Bravo, L., Roque, V. G., Brett, J., Dizon, R., and L'Azou, M. (2014). Epidemiology of dengue disease in the Philippines (2000-2011): a systematic literature review. *Public Library of Sciences Neglected Tropical Diseases [Electronic Resource]* ***8*****(11):** 3027.

Cao-Lormeau, V. M., Roche, c., Teissier, A., Robin, E., Berryl, A. L., Mallet, H. P., Sall, A. A. and Musso, D. (2014). Zika virus, French polynesia, south pacific 2013. *Emerging infectious diseases* ***20*****(6):** 1085-1086.

Capella-Gutierrez, S., Silla-Martinez, J. M. and Gabaldon, T. (2009). trimAl:a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* ***1;12*****(15):** 1972-1973.

Castro, J. S. M., and Oliveira, F. L. S. (2015). East / Central / South African Genotype Chikungunya Virus. *Emerging Infectious Diseases* ***21*****(5)**: 2013–2015.

Chadee, D. D. (2012). Studies on the post-oviposition blood-feeding behaviour of *Aedes aegypti* (L.) (Diptera: Culicidae) in the laboratory. *Pathogens and Global Health* ***10:*** 1179-1188.

Chaves, B. A., Orfano, A. S., Nogueira, P. M., Rodrigues, N. B., Campolina, T. B., Nacif-Pimenta, R., Pires, A., Junior, A.B. V., Paz, A. D. C., Vaz, E., Guerra, M.,Silva, B. M., de Melo, F. F., Norris, D. E., de Lacerda, M. V. G., Pimenta, P. F. P. and Secundino, N. F. C. (2018). Coinfection with Zika virus and Dengue virus results in preferential Zika virus transmision by vector bite to vertebrate host. *Journal of infectious diseases* ***218*****(4):** 563-571.

Chomczynski, P. M. K. (1995). Short technical report. Modification of the TRIZOL reagent procedure for isolation of RNA from Polysaccharide-and proteoglycan-rich sources. *Biotechniques* ***19*****(6):** 942–5.

Chretien, J.P., Anyamba, A., Bedno, S.A., Breiman, R.F., Sang, R., Sergon, K., Powers, A.M., Onyango, C.O., Small, J., Tucker, C.J., *et al.* (2007). Drought-associated chikungunya emergence along coastal East Africa. *American Journal of Tropical Medicine and Hygiene* ***76*****:** 405-407.

Coelho, G. C., Kraemer, U. G., Marianne, E. S., Kirsten, A. D., Adrian, Q. N. M., Freya, M. S., Christopher, M. B., Chester, G. M. and Roberta, G. C. (2015). The 1 global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. *Ecology, epidemiology and global health* ***4:*** 8347.

da Costa, C. F., dos Passos, R. A., Lima, J. B. P., Roque, R. A., de Souza Sampaio, V., Campolina, T. B., Nágila, F. C. S. and Paulo F. P. P. (2017). Transovarial transmission of DENV in *Aedes aegypti* in the Amazon basin: a local model of xenomonitoring. *Parasites & Vectors* ***10:*** 249.

Dash, P. K., Parida, M. M., Santhosh, S. R., Verma, S. K., Tripathi, N. K., Ambuj, S. and Sekhar, K. (2007). East Central South African genotype as the causative agent in reemergence of Chikungunya outbreak in India. *Vector Borne and Zoonotic Diseases* ***7*****(4):** 519–27.

Day, J. F. (2016). Mosquito oviposition behavior and vector control. *Insects* ***7*****(4)**: 65.

Edwards, F.W. (1941). Mosquitoes of the Ethiopian region III. *London, United Kingdom: London British Museum of Natural History*. 116-223.

Ellis, E. M., Neatherlin, J. C., delorey, M., Ochieng, M., Mohammed A. H., Mogeni, D. O., Hunsperger, E. Patta, S., Gikunju, S., Waiboic, L., Fields, B., Ofula, V., Konongoi, S. L., Torres-Velasquez, B., Marano, N., Sang, R., Margolis, H. S., Montgomery, J. M. and Tomashek, K. M. (2015). A house hold serosurvey to estimate the magnitude of a Dengue outbreak in Mombasa, Kenya, 2013. *Neglected Tropical Diseases* ***9*****(4):** 3733.

Fitch, W. M. (1970). Distinguishing homologous from analogous proteins. *Systematic Zoology* ***19*****:** 99–113.

Ganesan, V. K., Duan, B. and Reid, S. P. (2017). Chikungunya virus: Pathophysiology, Mechanism and Modelling. *Viruses* ***9*****(12):** 368.

Germa'n, A. G., Heisey, D. A. R., Espina, L. M., Stramer, S. L., and Rios, M. (2012). Short report: Phylogenetic analysis of dengue virus types 1 and 4 circulating in Puerto Rico and Key West, Florida, during 2010 epidemics. *American Journal of Tropical Medicine and Hygiene* ***87*****(3):** 548–553.

Gillies, M. T. and De Meillon, B. (1968). The *Anopheline* of Africa south of the Sahara (Ethiopian Zoogeographical Region). *Mosquito taxonomy inventory* ***54*****:** 1-343.

Göertz, G. P., Vogels, C. B. F., Geertsema, C., Koenraadt, C. J. M. and Pijlman, G. P. (2017). Mosquito co-infection with Zika and Chikungunya virus allows simultaneous transmission without affecting vector competence of *Aedes aegypti. Public Library of Science Neglected Tropical Diseases* ***10:*** 371-385.

Grandadam, M., Caro, V., Plumet, S., Thiberge, J., Souares, Y., Failloux, A., Tolou, H. J., Budelot, M., Cosserat, D., Leparc-Goffart, I. and Despres, P. (2011). Chikungunya virus, southeastern France. *Emerging infectioius diseases* ***17*****(15):** 910-913.

Grard, G., Caron, M., Mombo, I. M., Nkoghe, D., Ondo, S. M., Jiolle, D., Fontenille, D., Paupy, C. and Leroy, E. M. (2014). Zika virus in Gabon (Central Africa) - 2007: A new threat from *Aedes albopictus? Neglected Tropical Diseases **2***:8.

Grech, M. G., Ludueña-Almeida, F. and Almirón, W. R. (2010). Bionomics of *Aedes aegypti* subpopulations (Diptera_ Culicidae) from Argentina - Grech - 2010. *Journal of Vector Ecology* - Wiley Online Library.

Grossi-Soyster, E. N., Cook, E. A., de Glanville, W. A., Thomas, L. F., Krystosik, A. R., Lee, J., Wamae, C. J., Kariuki, S., Fevre, E. M. and La Beaud, A. D. (2017). Serological and spatial analysis of alphavirus and flavivirus prevalence and riks factors in a rural community in western Kenya. *Public Library of Sciences Neglected tropical diseases **11***(**10**)**:** e0005998.

Gubler, D. J. (2002). The Global Emergence/Resurgence of Arboviral Diseases As Public Health Problems. *Archives of Medical Research **33***(**4**)**:** 330–342.

Gubler, D. J., and Clark, G. G. (1995). Dengue/dengue hemorrhagic fever: the emergence of a global health problem. *Emerging Infectious Diseases*: ***1*(2):** 55–7.

Guzman, M. G., Halstead, S. B., Artsob, H., Buchy, P., Farrah, J., Gubler, D. J., Hunsperger, E., Kroeger, A., Margolis, H. S., Martinez, E., Nathan, M. B., Pelegrino, J. L., Simmons, C., Yoksan, S. and Peeling, R. W. (2010). Dengue: A continuing global threat. *Nature reviews microbiology* ***8*(12):** 7-16.

Harbach, R. E. (1988). The mosquitoes of the subgenus Culex in Southwestern Asia and Egypt (Diptera: Culicidae). *Control American Entomology Institution **24***: 240.

Harrington, L. C., Fleisher, A., Ruiz-Moreno, D., Vermeylen, F., Wa, C. V., Poulson, R. L. and Scott, T. W. (2014). Heterogeneous Feeding Patterns of the Dengue Vector, *Aedes aegypti*, on Individual Human Hosts in Rural Thailand. *Public Library of Science Neglected Tropical Diseases*. ***10*:** 1348-1370.

Harrison, C. J., and Langdale, J. A. (2006). A step by step guide to phylogeny reconstruction. *The plant journal **45:*** 561–572.

Hennig, W. (1966). Phylogenetic Systematics. Urbana, Illinois. *University of Illinois Press.*

Huang, Y. M. (2002). A pictorial key to the mosquito genera of the world, including subgenera of *Aedes* and *Ochlerotatus* (Diptera: Culicidae). *Journal of Korean nature **19***: 1-130.

Iwashita, H., Higa, Y., Futami, K., Lutiali, P. A., Njenga, S. M., Nabeshima, T. and Minakawa. N. (2018). Mosquito arbovirus survey in selected areas of Kenya: detection of insect-specific virus. *Tropical medicine and health **46***: 19.

Johnson, B. K., Musoke, S., Ochieng, D., Gichogo, A. Rees, P. H. (1982). Dengue-2 virus in Kenya. *Lancet* ***2*(8291):** 208-209.

Kajeguka, D. C., Kaaya, R. D., Mwakalinga, S., Ndossi, R., Ndaro, A., Chilongola, J. O. and Alifrangis, M. (2016). Prevalence of Dengue and Chikungunya virus infections in north-eastern Tanzania: a cross sectional study among participants presenting with malaria-like symptoms. *Biomedical central Infectious Diseases* ***16*****(1):** 183.

Kitching, I.J., Forey, P.L., Humphries, C.J. and Williams, D. M. (1998). Cladistics (2nd edn): The Theory and Practice of Parsimony Analysis. Oxford, UK. *Oxford University Press.*

Konongoi, L., Ofula, V., Nyunja, A., Owaka, S., Koka, H., Makio, A. and Sang, R. (2016). Detection of dengue virus serotypes 1, 2 and 3 in selected regions of Kenya: 2011–2014. *Journal of Virology* ***13:*** 182.

Kosasih, H., Alisjahbana, B., Nurhayati, de Mast, Q., Rudiman, I. F., Widjaja, S. and Porter, K. R. (2016). The Epidemiology, Virology and Clinical Findings of Dengue Virus Infections in a Cohort of Indonesian Adults in Western Java. *Public Library of Science Neglected Tropical Diseases* ***10:*** 1371.

Kraemer, M. U. G., Sinka, M. E., Duda, E. A., Mylne, A. Q. N., Shearer, F. M., Barker, C. M., Moore, C. G., Carvalho, R. G., Coelho, G. E., Bortel, W. V., Hendrickx, G., Schaffner, G., Elyazar, I. R., Teng, H., Brandy, O. J., Messina, J. P., Piggot, D. M., Scott, T. W., Smith, D. L., Wint, G. R., Golding, N. and Hay, S. I. (2015). The global distribution of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus*. *Science Data* ***2*****:**150035.

Kraus, H.,Weber, A., Appel, M., Enders, B., Isenberg, H. D., Schiefer, H. G, Slenczka, H. G., Graevenitz, A. V. and Zahner, H. (2003). Viral zoonoses. Zoonoses: Infectious diseases transmissable from animals to humans. Washington, USA: *American society of microbiology press* 3rd edition: 57-61.

Kumar, S., Mamidi, P., Kumar, A., Basantray, I., Bramha, U., Dixit, A., Maiti, P. K., Singh, S., Suryawanshi, A. R.and Chattopadhyay, S. (2015). Development of novel antibodies against non-structural proteins nsP1, nsP3 and nsP4 of Chikungunya virus: Potential use in basic research. *Archives of virology* ***160:*** 2749-2761.

Kumar, S., Stecher, G. and Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetic Analyser Version 7.0 for bigger datasets. *Molecular biology and evolution* ***33*****(7):** 1870-1874.

Kuno, G., Chang, G. J., Tsuchiya, K. R. and Karabatsos, N. C. C. (1998). Phylogeny of the genus flavivirus. *Journal of Virology* ***72*****(1):** 73–83.

La Beaud, A. D., Banda, T., Brichard, J., Muchiri, E. M., Mungai, P. L., Mutuku, G. M., Borland, E., Gildengorin, E., Pfeil, S., Yeng, C. Y., Long, K., Heise, M., Powers, A. M, Kitron, U. and King, C. (2015). High rates of O'nyong Nyong and Chikungunya virus transmission in coastal Kenya. *Neglected tropical diseases* ***9*****(4):** 3674.

Lanciotti, R. S., and Valadere, A. M. (2014). Transcontinental movement of Asian Genotype Chikungunya virus. *Emerging Infectious Diseases* ***20*****(8):** 1400-1402.

Lanciotti, R. S., Calisher, C. H., Gubler, D. J., Chang, G. J. and Vorndam, A. V. (1992). Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. *Journal of Clinical Microbiology* ***30*****(3):** 545–51.

Lardeux, F., Sechan, Y. and Faaruia, M. (2002). Evaluation of insecticides impregnated baits for control of mosquito larvae in land crab burrows on french polynesian atolls. *Journal of medical entomology* ***39*****(4):** 658-661.

Le Coupanec, A., Tchankouo-Nguetcheu, S., Roux, P., Khun, H., Huerre, M., Morales-Vargas, R. and Choumet, V. (2017). Co-infection of mosquitoes with Chikungunya and Dengue viruses reveals modulation of the replication of both viruses in midguts and salivary glands of *Aedes aegypti* mosquitoes. *International Journal of Molecular Sciences* ***18*****(8)**: 1708.

Leparc-Goffart, I., Nougairede, A., Cassadou, S., Prat, C. and de Lamballerie, X. (2014). Chikungunya in the Americas. *Lancet* ***383*****(9916):** 514.

Lindsey, N. P., Lehman, J. A., Staples, J. E. and Fischer, M. (2014). West Nile virus and other arboviral diseases - United States, 2013. *Morbidity and Mortality Weekly Report*. 12997.

Lutomiah, J., Barrera, R., Makio, A., Mutisya, J., Koka, H., Owaka, S. and Sang, R. (2016). Dengue Outbreak in Mombasa City, Kenya, 2013–2014: Entomologic Investigations. *Public Library of Sciences Neglected Tropical Diseases* ***10*****(10):** 2013–2014.

Lynch Arribalzaga. (1891). Genus *Ochlerotatus* Lynch Arribalzaga. Mosquito taxonomy inventory.

Martina, B. E. E., Koraka, P. and Osterhaus, A. D. M. E. (2009). Dengue virus pathogenesis: An integrated view. *Clinical microbiology reviews* ***22*****(4):** 564-581.

Mattingly, P. F. (1967). Taxonomy of *Aedes Aegypti* and Related Species. *Bulletin of the World Health Organization* ***36*****:** 552.

Mbaika, S., Lutomiah, J., Chepkorir, E., Mulwa, F., Khayeka-Wandabwa, C., Tigoi, C., Oyoo-Okoth, E., Mutisya, J., Ng'ang'a, Z., and Sang, R. (2016). Vector competence of *Aedes aegypti* in transmitting Chikungunya virus: effects and implications of extrinsic incubation temperature on dissemination and infection rates. *Journal of virology* ***13*****:** 114.

McClelland, G. A. H. (1960). A prelimnary study of the genetics of abdominal color variations in *Aedes aegypti* (L.) (Diptera: Culicidae). *Annals of Tropical Medicine and Parasitology* ***54*****:** 305–320.

Meigen, J. W. (1818). Genus *Aedes* Meigen. Mosquito taxonomy inventory.

Minard, G., Tran Van, V., Tran, F. H., Melaun, C., Klimpel, S., Koch, L. K. and Valiente Moro, C. (2017). Identification of sympatric cryptic species of *Aedes albopictus* subgroup in Vietnam: new perspectives in phylosymbiosis of insect vector. *Parasites and Vectors* ***10: 276.***

Moore, M., Sylla, M., Goss, L., Burugu, M. W., Sang, R., Kamau, L. W. and Sharakova, M. (2013). Dual African Origins of Global *Aedes aegypti* s . l . Populations Revealed by Mitochondrial DNA. *Neglected Tropical Diseases* ***7*****(4):** 2175.

Moro, M. L., Gagliotti, C., Silvi, G., Angelini, R., Sambri, V., Rezza, G. and Seyler, T. (2010). Chikungunya Virus in North-Eastern Italy : A Seroprevalence Survey. *American journal of Tropical Medicine and Hygiene* ***82*****(3):** 508–511.

Murray, N. E. A., Quam, M. B., and Wilder-Smith, A. (2013). Epidemiology of dengue: Past, present and future prospects. *Clinical Epidemiology* ***5:*** 299-309.

Ndenga, B. A., Mutuku, F. M., Ngugi, H. N., Mbakaya, J. O., Aswani, P., Musunzaji, P. S., Vulule, J., Mukoko, D., Kitron, U. and La Beaud, A. D. (2017). Characteristics of *Aedes aegypti* adult mosquitoes in rural and urban areas of western and coastal Kenya. *Public library of sciences one* ***12*****(12):** e0189971.

Ngala, Jonathan. (2020). RNA sequences for Aedes species, Dengue and Chikungunya viruses, v3, Dryad, Dryad, Dataset, https://doi.org/10.5061/dryad.nk98sf7r6

Ngoi, C. N., Price, M. A., Fields, B., Bonventure, J., Ochieng, C., Mwashigadi, G., Hassan, A. S., Thiong'o, A. N., Micheni, M., Mugo, P., Graham, S. and Sanders, E. J. (2016). Dengue and Chikungunya virus infections among youth febrile adults evaluated for acute HIV-1 infection in coastal Kenya. *Public library of sciences one* ***11*****(12):**167508.

Ngugi, H. N., Mutuku, F. M., Ndega, B. A., Mbakaya, J. O., Aswani, P., Musunzaji, P. S., Irungu, L. W., Vulule, J., Mukoko, D., Kitron, U. and La Beaud, A. D. (2017). Characterization and productivity profiles of *Aedes aegypti* (L.) breeding habitats across rural and urban landscapes in western and Coastal Kenya. *Parasites and vectors* ***10:*** 331.

Njenga, K. M., Nderitu, L., Ledermann, J. P., Ndirangu, A., Logue, C. H., Kelly, C. H. L. and Powers, A. M. (2008). Tracking epidemic Chikungunya virus into the Indian Ocean from East Africa. *The Journal of General Virology* ***89*****(11):** 2754–60.

Normile, D. (2013). Tropical medicine. Surprising new Dengue virus throws a spanner in disease control efforts. *Science* ***342:***415.

Ochieng, C., Lutomiah, J., Makio, A., Koka, H., Chepkorir, E., Yalwala, S. and Sang, R. (2013). Mosquito-borne arbovirus surveillance at selected sites in diverse ecological zones of Kenya . *Journal of virology* ***10:*** 140.

Ochieng, C., Ahenda, P., Vittor, A. Y., Nyoka, R., Gikunju, S., Wachira, C., Waiboci, L., Umuro, M., Kim, A. A., Nderitu, L.,Juma, B., Montgomery, J. M., Breiman, R. F. and Fields, B. (2015). Seroprevalence of Infections with Dengue, Rift valley Fever and Chikungunya viruses in Kenya, 2007. *Public Library of Science One* **10(7):**e0132645.

Paranjape , S. M and Harris, E. (2010). Control of Dengue virus translation and replication. *Current topics in microbiology and immunology* ***338:*** 15-34.

Paupy, C., Le Goff, G., Brengues, C., Guerra, M. and Revollo, J. (2012). Genetic structure and phylogeography of *Aedes aegypti*, the Dengue and Yellow-fever mosquito vector in Bolivia. *Infection Genetics and Evolution* ***12*****:** 1260–1269.

Petersen, L. R., Powers, A. M., Weaver, S. C., Forrester, N. L., Zanluca, C., Melo, V. C. A. and Olowokure, B. (2016). Chikungunya: epidemiology. *Vector Borne and Zoonotic Diseases* ***5:*** 7171.

Petitdemange, C., Wauquier, N. and Vieillard, V. (2015). Control of immunopathology during Chikungunya virus infection. *Journal of Allergy and Clinical Immunology* ***135:*** 846-855.

Pevsner, J. (2015). Bioinformatics and Genomics 3rd Edition. *Science* 256-1053.

Rambaut, A. (1996). Se-Al: Sequence Alignment editor. Available at http://evolve.zoo.ox.ac.uk/software.html?id=seal.

Ranjit, S. and Kissoon, N. (2011). Dengue hemorrhagic fever and shock syndromes. *Pediatric Critical Care Medicine : A Journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies* ***12*****(1):** 90–100.

Reinert, J. F. (2000). New classification for the composite genus *Aedes* (diptera : culicidae : aedini), elevation of subgenus ochlerotatus to generic rank , reclassification of the other subgenera . And notes on certain subgenera and species. *Journal of American Mosquito Control Association* ***16*****(3):** 175–188.

Renault, P., Solet, J. L., Sissoko, D., Balleydier, E., Larrieu, S., Filleul, L. and Pierre, V. (2007). A major epidemic of Chikungunya virus infection on Réunion Island, France, 2005-2006. *American Journal of Tropical Medicine and Hygiene* ***77*****(4):** 727–731.

Rico-Hesse, R. (2009). Dengue virus markers of virulence and pathogenecity. *Future virology* ***4*****(6):** 581-589.

Rodenhuis-Zybert, I. A., Van der schaar, H. M., da Silva Voorham, J. M., van der Endee-Metselaar, H., Lei, H. Y., Wilschut, J. and Smit, J. M. (2010). Immature Dengue virus: a veiled pathogen? *Public library of sciences Pathogens* ***6*****(1):** 718.

Roth, A., Mercier, A., Lepers, C., Hoy, D., Duituturaga, S., Benyon, E. and Souarès, Y. (2014). Concurrent outbreaks of Dengue, Chikungunya and Zika virus infections – an unprecedented epidemic wave of mosquito-borne viruses in the Pacific 2012 – 2014. *www.eurosurveillance.org* ***19*****(41):** 1–8.

Rougeron, V., Sam, I. C., Caron, M., Nkoghe, D., Leroy, E. and Roques, P. (2015). Chikungunya, a paradigm of neglected tropical disease that emerged to be a new health global risk. *Journal of clinical virology* ***64*****:**144-152.

Rückert, C., Weger-Lucarelli, J., Garcia-Luna, S. M., Young, M. C., Byas, A. D., Murrieta, R. A. and Ebel, G. D. (2017). Impact of simultaneous exposure to arboviruses on infection and transmission by *Aedes aegypti* mosquitoes. *Nature Communications* ***8:*** 15412.

Sang, R., Arum, S., Chepkorir, E., Mosomtai, G., Tigoi, C., Sigei, F., Lwande, O., Landmann, T., Affognon, H., Ahlm, C. and Evander, M. (2017). Distribution and abundance of key vectors of Rift Valley fever and other arboviruses in two ecologically distinct Counties in Kenya. *Public Library of Science Neglected Tropical Diseases* ***11*****(2):** e0005341.

Sang, R., Kioko, E., Lutomiah, J., Warigia, M., Ochieng, C., O'Guinn, M. and Richardson, J. (2010). Rift Valley fever virus epidemic in Kenya, 2006/2007: The entomologic investigations. *American Journal of Tropical Medicine and Hygiene* ***83*****(2):** 28–37.

Sanger, F., Nicklen, S. and Coulson, A. R. (1977). DNA sequencing with chain terminating inhibitors. *Proceedings of the National Academy of Sciences of the United States of America* ***74*****(12):** 5463–5467.

Seabloom, E. W., Hosseini, P. R., Power, A. G. and Borer, E. T. (2009). Diversity and composition of viral communities: coinfection of barley and cereal yellow dwarf viruses in California grasslands. *The American society of naturalis*t ***173*****:**3.

Sergon, K., Njuguna, C., Kalani, R., Ofula, V., Onyango, C., Konongoi, L. S. and Breiman, R. F. (2008). Seroprevalence of Chikungunya Virus (CHIKV) Infection on Lamu Island, Kenya, October 2004. *American Journal of Tropical Medicine and Hygiene* ***78*****(2):** 333–337.

Sharp, T. M., Roth, N. M., Torres, J., Ryff, K. R., Perez Rodriguez, N. M., Mercado, C., Pilar, D. P., M . D., Ramos, M., Phillips, R., Lozier, M., Arriola, C S., Johansson, M., Hunsperger, E., Munoz-Jordan, J. L., Margolis, H. S. and Garcia, B. R. (2014). Chikungunya cases identified through passive surveillance and household investigations--Puerto Rico, May 5-August 12, 2014. *Public Library of Sciences on Neglected Tropical Diseases Morbidity and Mortality Weekly Report* ***63*****(48):** 1121-1128.

Shepard, J. J. 1., Andreadis, T. G. and Vossbrinck, C. R. (2006). Molecular phylogeny and evolutionary relationships among mosquitoes (Diptera: Culicidae) from the northeastern United States based on small subunit ribosomal DNA (18S rDNA) sequences. *Journal of Medical Entomology* ***43*****(3):** 443–54.

Sievers, F. and Higgins, D. G. (2014). Clustal omega. *Current protocols in Bioinformatics* ***12*****(48):** 3.13.1-16.

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* ***1;30*****(9):** 1312-1313.

Sutherland, L. J., Cash, A. A., Huang, Y.-J. S., Sang, R. C., Malhotra, I., Moormann, A. M. and La Beaud, A. D. (2011). Serologic evidence of arboviral infections among humans in Kenya. *The American Journal of Tropical Medicine and Hygiene* ***85*****(1):** 158–61.

Tabachnick, W. J, Wallis, G. P., Aitken, T. H. G., Miller, B. R. and Amato, G. D. (1985). Oral Infection of *Aedes-Aegypti* with Yellow-Fever Virus - Geographic- Variation and Genetic Considerations. *American Journal of Tropical Medicine and Hygiene* ***34*****:** 1219–1224.

Tamura, K. N. M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology of Evolution* ***10*****:** 512–26.

Thiberville, S. D., Boisson, V., Gaudart, J., Simon, F., Flahault, A. and de Lamballerie, X. (2013a). Chikungunya Fever: A Clinical and Virological Investigation of Outpatients on Reunion Island, South-West Indian Ocean. *Public Library of Sciences on Neglected Tropical Diseases* ***9*****(3):** 345-370.

Thiberville, S. D., Moyen, N., Dupuis-Maguiraga, L., Nougairede, A., Gould, E. A., Roques, P. and de Lamballerie, X. (2013b). Chikungunya fever: Epidemiology, clinical syndrome, pathogenesis and therapy. *Antiviral Research **7***(**1**)**:** 1016.

Tigoi, C., Lwande, O., Orindi, B., Irura, Z., Ongus, J. and Sang R. (2015). Seroepidemiology of selected arboviruses in febrile patients visiting selected health facilities in the lake/ river basin areas of lake baring, lake naivasha and tana river, Kenya. *Vector-borne and zoonotic diseases **15***(**2**)**:** 1686.

Trpis, M. and Hausermann, W. (1986). Dispersal and other population parameters of *Aedes aegypti* in an African village and their possible significance in epidemiology of vector-borne diseases. *American journal of Tropical Medicine and Hygiene **35(6):*** 1263-79.

Vazeille, M., Mousson, L., Martin, E. and Failloux, A.-B. (2010). Orally co-Infected *Aedes albopictus* from La Reunion Island, Indian Ocean, can deliver both Dengue and Chikungunya infectious viral particles in their saliva. *Public Library of Sciences on Neglected Tropical Diseases Neglected Tropical Diseases **4***(**6**): 706.

Volk, S. M., Chen, R., Tsetsarkin, K. A., Adams, A. P., Garcia, T. I., Sall, A. A. and Weaver, S. C. (2010). Genome-Scale Phylogenetic Analyses of Chikungunya Virus Reveal Independent Emergences of Recent Epidemics and Various Evolutionary Rates. *Journal of Virology **84***(**13**)**:** 6497–6504.

Wang, B., Li, Y., Feng, Y., Zhou, H., Liang, Y., Dai, J. and Xia, X. (2015). Phylogenetic analysis of Dengue virus reveals the high relatedness between imported and local strains during the 2013 Dengue outbreak in Yunnan, China: a retrospective analysis. *Biomedical Centre for Infectious Diseases, **15***(**1**)**:** 142.

Weeratunga, P., Rodrigo, C., Fernando, S. D. and Rajapakse, S. (2017). Control methods for *Aedes albopictus* and *Aedes aegypti*. *Cochrane database of systematic reviews* 2017-8.

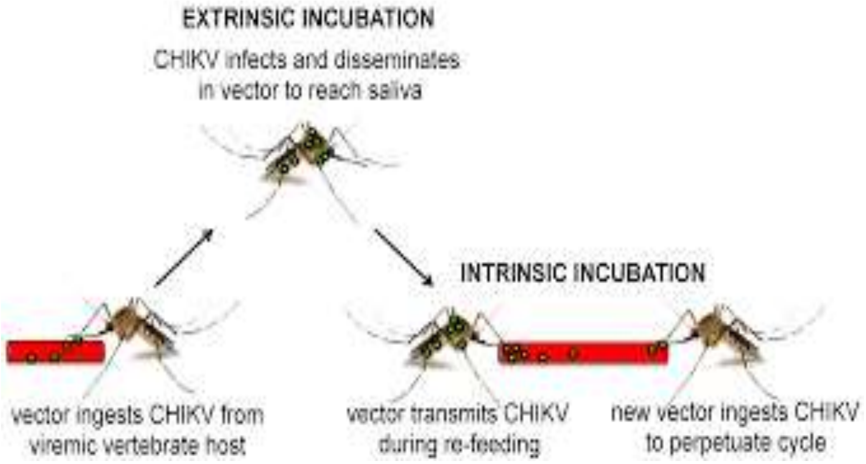
Yoon, I. K., Alera, M. T., Lago, C. B., Tac-An, I. A., Villa, D., Fernandez, S. and Srikiatkhachorn, A. (2015). High Rate of Subclinical Chikungunya Virus Infection and Association of Neutralizing Antibody with Protection in a Prospective Cohort in The Philippines. *Public Library of Sciences on Neglected Tropical Diseases Neglected Tropical Diseases **9***(**5**)**:** 3764-3771.

Yung, C., Lee, K., Thein, T., Tan, L., Gan, V. C., Wong, J. G. X., Lye, L. and Leo, Y. (2015). Dengue serotype-specific differnces in clinical manifestation, laboratory parameters and risk of severe diseases in adults, Singapore. *American journal of Tropical medicine and Hygiene **92***(**5**)**:** 999-1005.

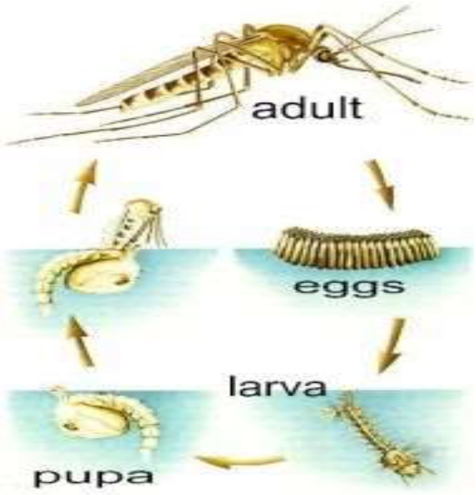
Zeller, H., Bortel, W. V. and Sudre, B. (2016). Chikungunya: Its history in Africa and Asia and its spread to new regions in 2013-2014. *The journal of infectious diseases **214***(**5**)**:** 436-440.

APPENDICES

Appendix 1: Incubation of arboviruses in *Aedes* mosquitoes (Bravo *et al.*, 2014)



Appendix 2: Life cycle of *Aedes* mosquitoes (Day, 2016)

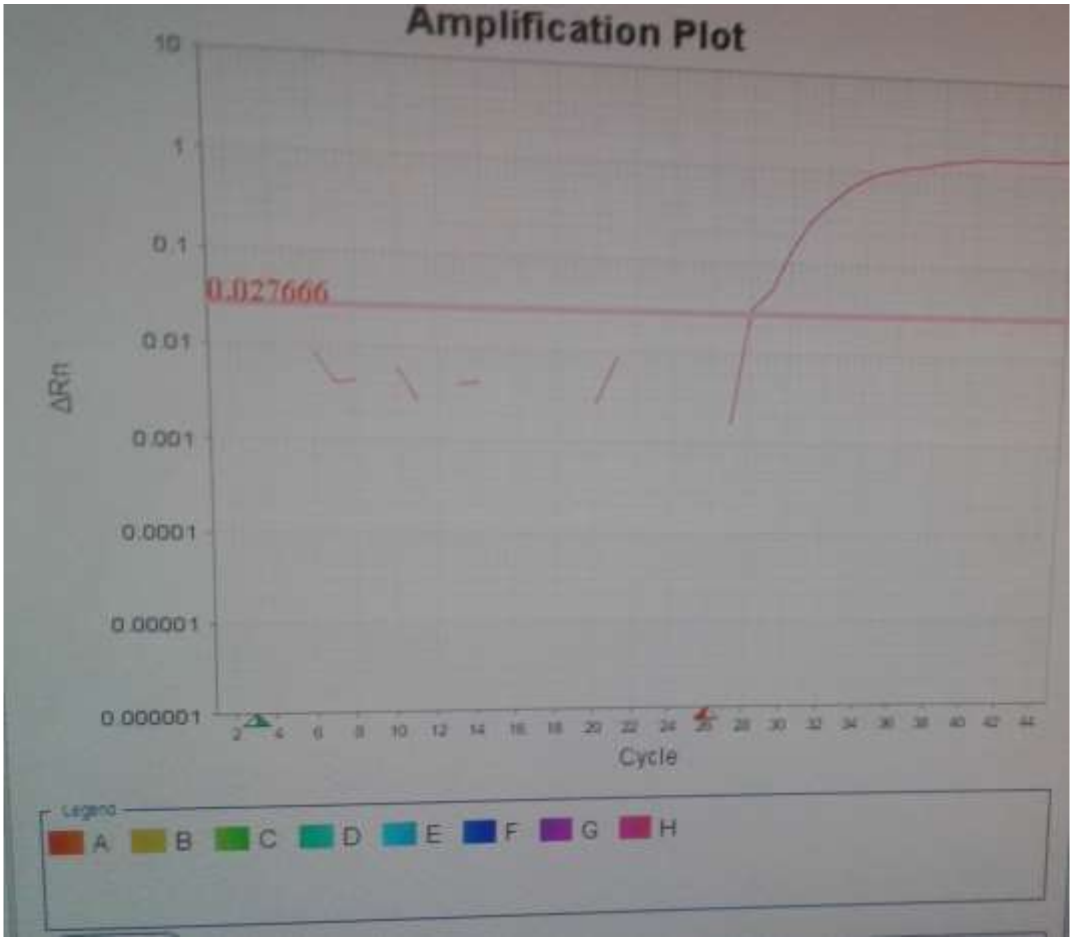


Appendix 3: *Aedes* mosquitoes in sentinel sites

County/ Site name	**GPS coordinates Latitude (S)**	**Longitude (E)**	**No. of mosquitoes collected Dry season**	**Wet season**	**Totals**
Mpeketoni	02.2696° S	40.9006° E	2,860	4,674	7,534
Lamu			**2,860**	**4,674**	**7,534**
Malindi	03.3425° S	40.0274° E	2,858	2,820	5,678
Watamu	03.3425° S	40.0274° E	220	300	520
Kilifi	03.62940° S	039.86605° E	1,484	1,620	3,104
Rabai	03.92861° S	039.57248° E	657	1020	1677
Mazera	03.97152° S	039.54900° E	387	280	667
Kilifi			**5,606**	**6,040**	**11,646**
Shimo la tewa	03.95776°S	039.74234°E	900	1,610	2,510
Nyali	04.02749° S	039.69432° E	634	930	1,564
Tononoka	04.05182° S	039.67141° E	420	600	1,020
Tudor	04.03235° S	039.66100° E	187	260	447
Port Reitz	04.03744° S	039.60837° E	638	940	1,578
Mombasa			**2,779**	**4,340**	**7,119**
Kwale	04.17489°S	039.45586° E	1,388	1,540	2,928
Tiwi	04.23488° S	039.57470° E	396	504	900
Diani	04.27956° S	039.56773° E	1,724	2,800	4,524
Msambweni	04.4653°S	039.4813°E	202	360	562
Lunga lunga	04.55573°S	039.12374° E	640	780	1,420
Vanga	04.66064° S	039.2166° E	187	400	587
Kwale			**4,537**	**6384**	**10,921**
Grand totals			**15,782**	**21,438**	**37,220**

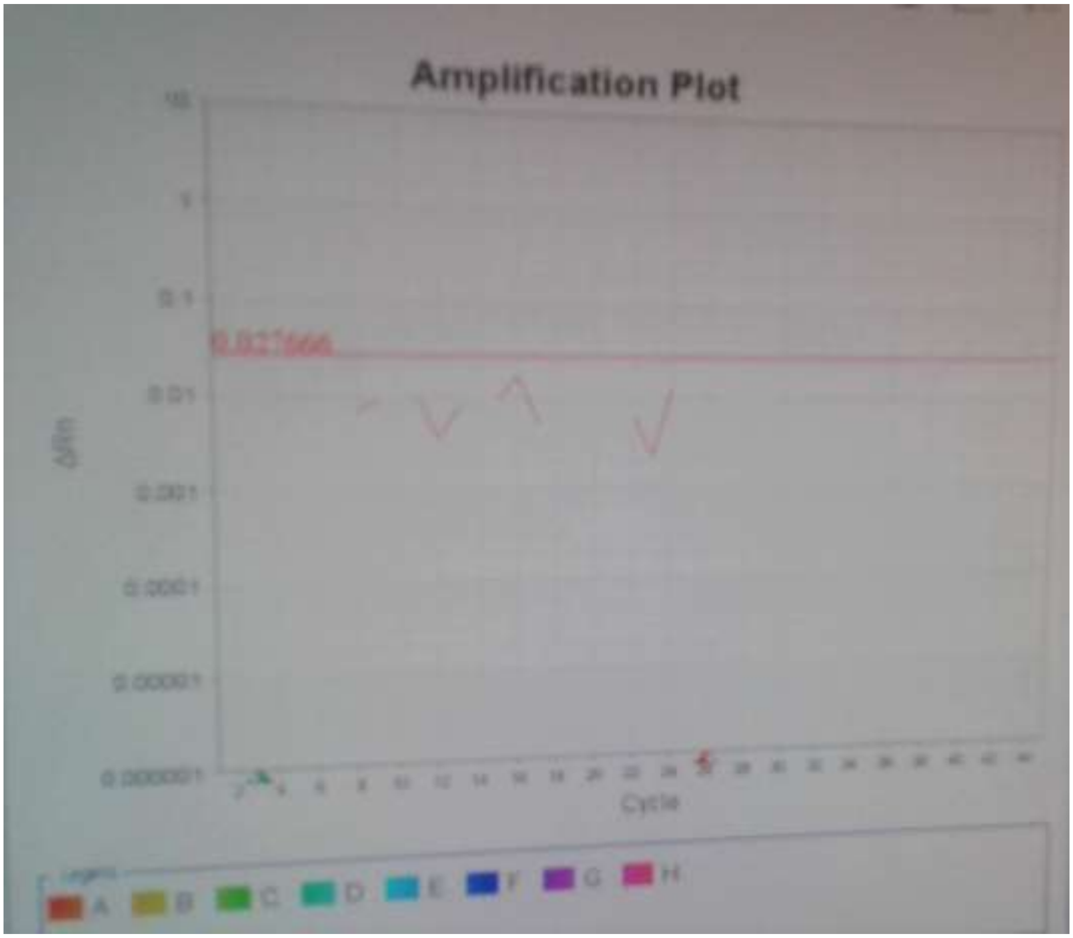
Appendix 4: Multiplex qPCR display curves

(a) Positive control fluorescent display curve



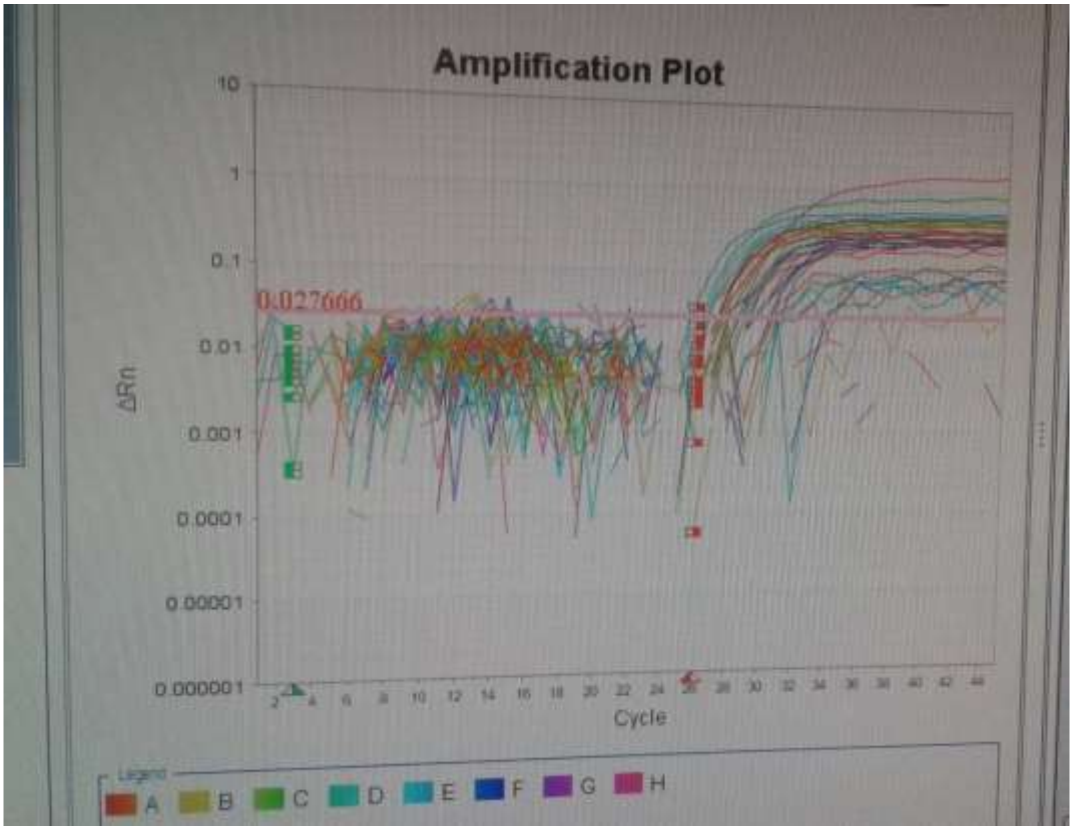
Legend: The colored boxes on the legend: red indicates positive control; Pink represents negative control and the middle boxes represent the samples under test. The positive sample was amplified at threshold cycle (CT) value of 26.

(b) Negative control fluorescence display curves



Legend: The colored boxes on the legend: red indicates positive control; Pink represents negative control and the middle boxes represent the samples under test. No amplification occurred for this negative sample with reaction rate of an amplitude of <0.027666.

(c) Controls and samples fluorescence display curves



Legend: Amplification of the samples and controls occurred at a CT value of 26. The colored boxes on the legend: red indicates positive control; Pink represents negative control and the middle boxes represent the samples under test.

Appendix 5: Primers and probes for flaviviruses and alpha viruses

Primers and Probes	Sequence	Tm ^{o}
Flavivirus		
Flavi allS (Forward Primer)	5’-TACAACATgATggggAARAgAgARAA-3’	53.8
Flavi all AS2 (Reverse Primer)	5’-gTgTCCCAgCCNgCKgTgTCATCWgC-3’	
Flavi all AS4 (Reserve Primer)	5’-gTgTCCCAGCCNgCKgTRTCRTC-3’	80.4
$3P_i$ (Probe)	FAM-Tg+gTWYATgT+ggYTNg+gRgC-NFQ-MGB	50.3
$3P_{ii}$ (Probe)	FAM-CCgTgCCATATggTATATgTggCTgggAgC-NFQ-MGB	
$3P_{iii}$ (Probe)	FAM-TTTCTggAATTTgAAgCCCTgggTTT-NFQ-MGB	
Pan-alphavirus		
F2A (Forward Primer)	5’- ATGATGAARTCIGGIATGTTYYT-3’	
R2A (Reverse Primer)	5’-ATYTTIACTTCCATGTTCATCCA-3’	
R3A (Reverse Primer)	5’-ATYTTIACTTCCATRTTCARCCA-3’	
R4A (Reverse Primer)	5’-ATYTTIACTTCCATGTTGACCCA-3’	
ATTO425 (Probe)	- AT+GTT+GTC+GT+CIC+CIAT-BHQ1/LNA	
Forward Primers	5’-TTCTTTGCTTCTGATACCCTCTGT-3’	
Reserve Primers	5’-GTTCCACTTCCTTGCATCATCTG-3’	
Probe	FAM-TTGCACAAGTCCACACAGGCCCCT-TAMRA	

Appendix 6: Supplementary files (https://doi.org/10.5061/dryad.nk98sf7r6)

Nucleotide sequences in FASTA format for mtNADH-4 gene of *Aedes species*.

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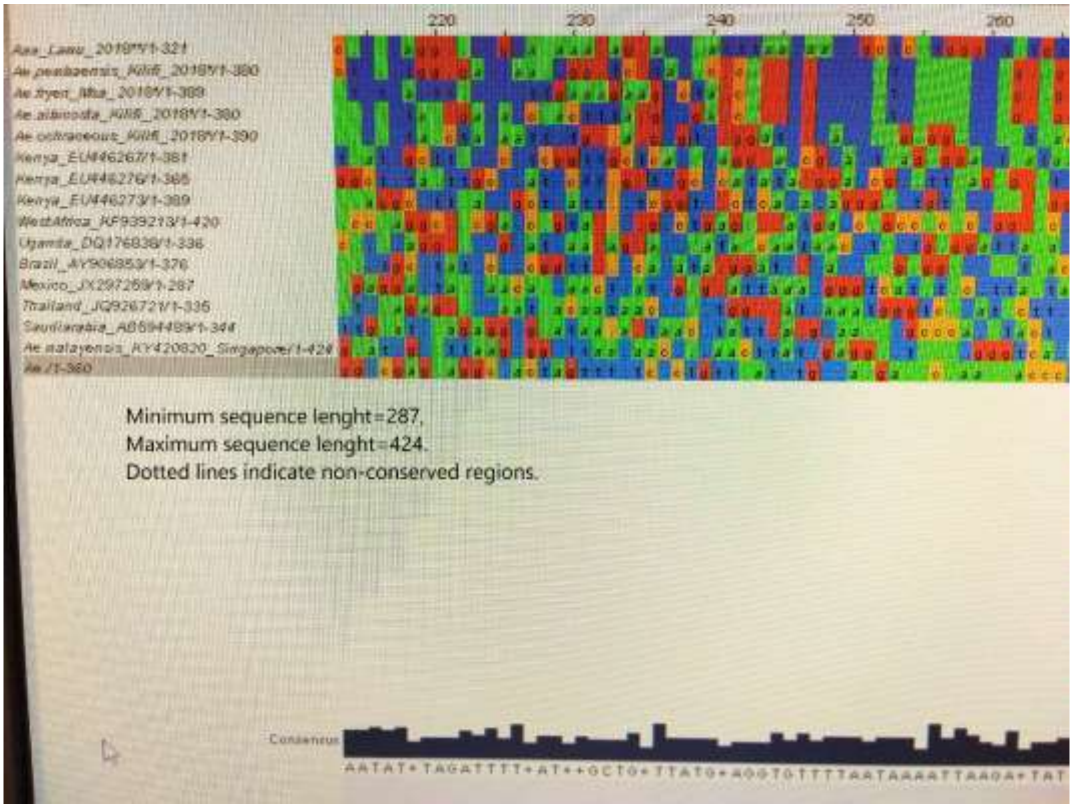
A section of Jalview for *Aedes* sequences



Minimum sequence lenght=287.
Maximum sequence lenght=424.
Dotted lines indicate non-conserved regions.

T-Coffee alignment and scores for *Aedes* sequences

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 Build 507)
Ngalachome
SCORE=610
BAD AVG GOOD
Aaf_Lamu_2018*    :  63
Aaf_Kilifi_2018   :  63
Aaf_Mombasa_201   :  64
Aaf_Kwale_2018*   :  64
Aaa_Lamu_2018**   :  55
Aaa_Kilifi_2018   :  68
Aaa_Mombasa_201   :  69
Aaa_Kwale_2018*   :  69
Ae.pembaensis_K   :  56
Ae.pembaensis_M   :  62
Ae.pembaensis_K   :  62
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Ae.tricholabis_   :  53
Ae.tricholabis_   :  53
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Ae.fulgens_Msa_ : 34
Ae.albicosta_Ki : 35
Ae.ochraceous_K : 34
Ae.ochraceous_L : 45
Ae.mcintoshi_Ki : 44
Ae.mcintoshi_La : 45
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Kenya_EU446276 : 65
Kenya_EU446273 : 63
Kenya_EU446271 : 63
WestAfrica_EF56 : 70
WestAfrica_KF93 : 30
Global_AF203356 : 63
Uganda_DQ176838 : 69
Brazil_AY906853 : 67
Mexico_JX297259 : 67
Thailand_JQ9267 : 70
Saudiarabia_AB5 : 47
cons : 61

Nucleotides sequences FASTA format for NSP5 gene of Dengue virus

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>Dengue1_Mombasa_2018
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>Dengue1_Kilifi_2018
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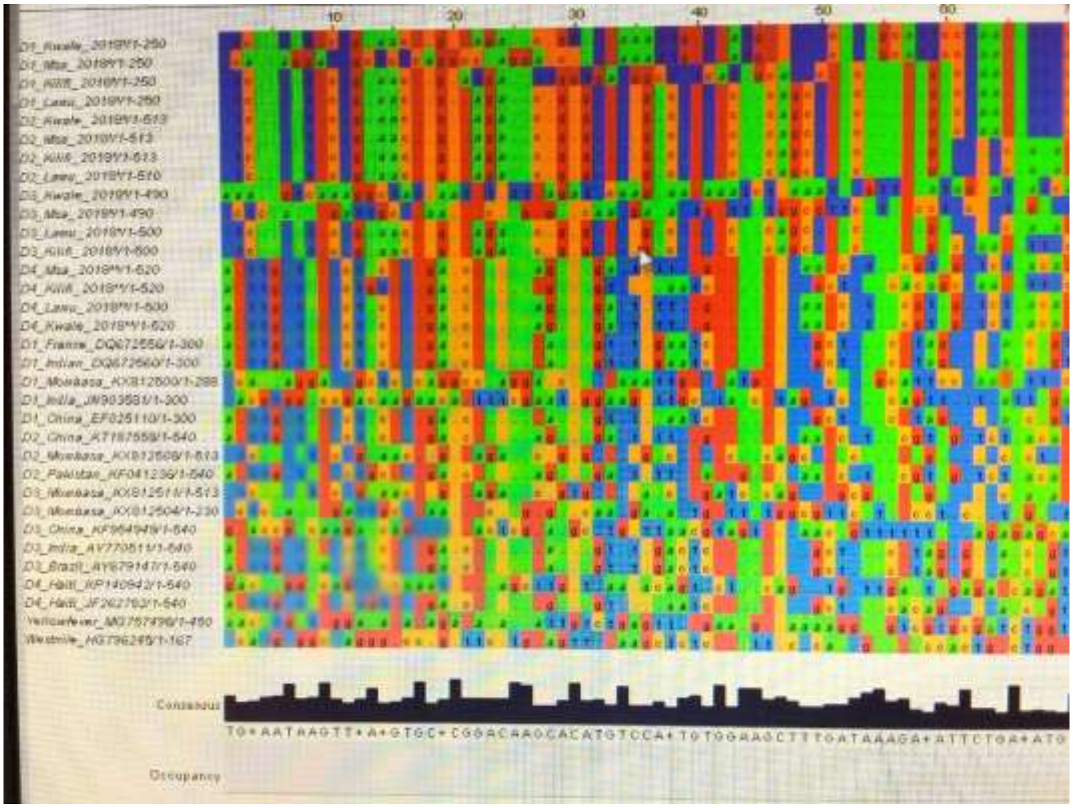
A section of Jalview for Dengue virus sequences



T-Coffee alignment and scores for Dengue virus sequences

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 Build 477)
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CPU TIME:0 sec.
SCORE=545
BAD AVG GOOD
D3_Kwale_2018     :  48
D3_Lamu_2018      :  61
D1_Kwale_2018     :  55
D1_Lamu_2018      :  59
D1_Kilifi_2018    :  61
D2_Kilifi_2018    :  62
D2_Lamu_2018      :  63
D2_Kwale_2018     :  64
D3_Kilifi_2018    :  63
D4_MOMBASA_2018   :  48
cons              :  54
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Nucleotides sequences FASTA format for NSP1 gene of Chikungunya virus

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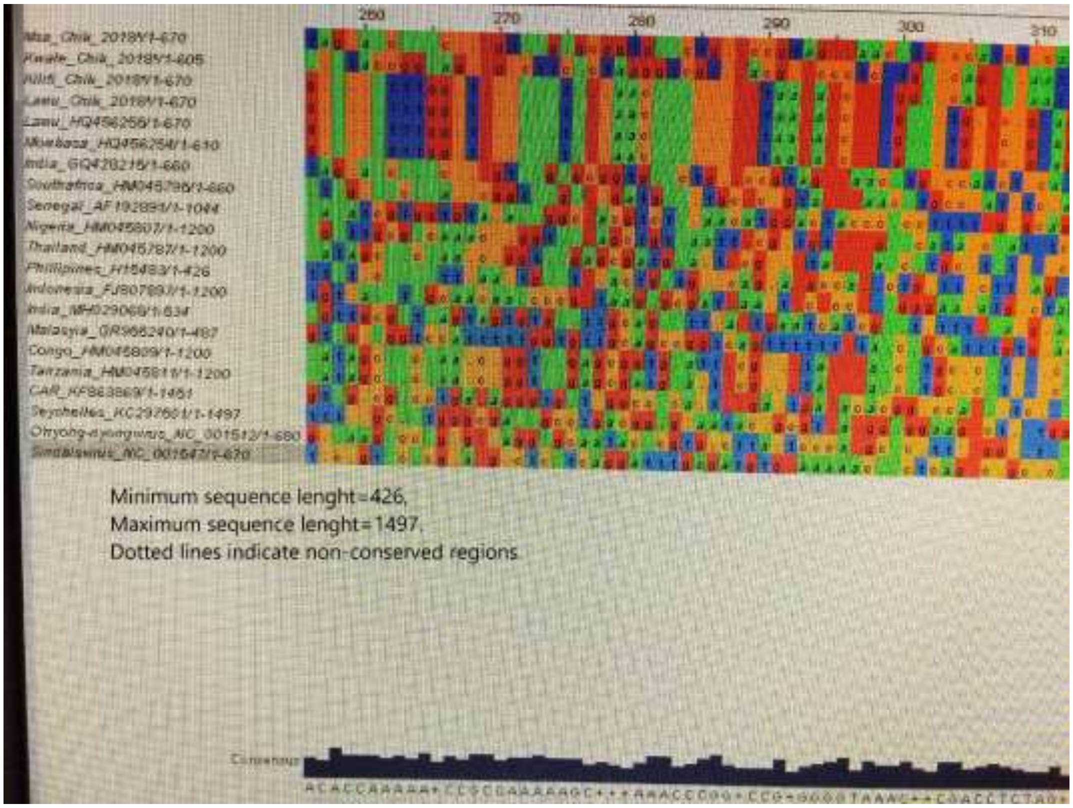
A section of Jalview for Chikungunya virus sequences



Minimum sequence lenght=426.
Maximum sequence lenght=1497.
Dotted lines indicate non-conserved regions

T-Coffee alignment and scores for Chikungunya virus sequences

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 Revision d625267 - Build 507)
ngalachome
SCORE=699
BAD AVG GOOD
Kwale_Chik_2018   :  72
Mombasa_Chik_20   :  63
Kilifi_Chik_201   :  75
lamu_Chik_2018*   :  78
Lamu_HQ456255     :  79
Mombasa_HQ45625   :  81
India_GQ428215    :  79
Southafrica_HM0   :  79
Senegal_AF19289   :  59
Nigeria_HM04580   :  82
Thailand_HM0457   :  79
Phillipines_H15   :  34
Indonesia_FJ807   :  79
India_MH029068    :  29
Malasyia_GR9552   :  41
Congo_HM045809    :  79
Tanzania_HM0458   :  79
CAR_KF863869      :  30
Seychelles_KC29   :  28
cons              :  69
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