TRANSMISSION DYNAMICS OF ARBOVIRUSES ALONG THE KENYAN COAST

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Transmission Dynamics of Arboviruses along the Kenyan Coast

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DECLARATION

This thesis is my original work and has not been submitted for a degree in any other University

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DEDICATION

This thesis is specially and specifically dedicated to my late parents; dad Peter Karuitha and mum Virginia Muthoni Karuitha, my late mother in-law Margaret Ng'ang'a, my daughter Michal Naipanoi, my son Malik Lemayian and everyone else who has played role in my career development.

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ABBREVIATIONS AND ACRONYMS

BSL2	Biosafety Level 2
BG	Biogents Mosquito Trap
cDNA	Complementary Deoxyribonucleic Acid
CDC	Center for Disease Control and Prevention
CHIKV	Chikungunya Virus
CO_2	Carbon Dioxide
DENV	Dengue Virus
^oC	Degree Celcius
dNTP	Deoxynucleotide Triphosphate
JKUAT	Jomo Kenyatta University and Agriculture
KEMRI:	Kenya Medical Research Institute
MEM	Minimum Essential Medium
PVC	Polyvinyl Chloride
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcription - Polymerase Chain Reaction
YFV	Yellow Fever Virus
VHF	Viral Hemorrhagic Fever
ZIKV	Zika Virus

ABSTRACT

Arboviruses are arthropod-borne viruses transmitted to humans and animals primarily through the bites of infected insects, mainly mosquitoes. While most arboviral infections are self-limiting, some can be life-threatening. Recent outbreaks have been reported along Coastal and northeastern Kenya. Understanding the factors influencing the occurrence and spread of these viruses including vector population density, mosquito feeding behavior, transovarial transmission, and the link between sylvatic and urban transmission cycles is crucial for developing effective control measures to prevent future outbreaks. This study employed a cross-sectional design to investigate the transmission dynamics of arboviruses in four urban areas, two forested areas, and two peri-urban areas along coastal Kenya. It also aimed establish the link between sylvatic and urban transmission cycles to better understand the factors contributing to arboviral outbreaks in urban settings. Mosquito eggs, larvae, and adult were collected across the study sites namely Mombasa Islands, Changamwe, Likoni and Nyari (urban areas), Haller Park and Arabuko Sokoke forest (forest areas) and Bamburi and Gede (peri-urban areas). The eggs were collected using ovipositor cups targeting aedes mosquitoes and dispensed in larval trays for hatching and together with larvae collected using dipper and pipetting methods reared to adults. Adult mosquitoes were collected using Center for Disease Control and Prevention (CDC) resting traps, Carbon dioxide ($CO_{2)}$-baited CDC light traps, and Biogents' mosquito traps (BG sentinel traps). All mosquitoes were identified morphologically to species level using taxonomic keys. Identified mosquitoes were pooled into pools of 25 based on species, sex and collection date and screened for arboviruses by both cell culture and Reverse Transcription-Polymerase Chain reaction (RT-PCR). Blood meal analysis from engorged females was conducted by amplifying the 12S mitochondrial ribosomal gene, followed by sequencing and BLAST analysis in GenBank to identify blood meal source. A total of 13,009 larvae were collected from 17 different habitats along coastal Kenya with discarded tires being a major breeding site that contributed to higher larval populations. Approximately 4,735 adult mosquitoes, representing 19 species, were collected from peri-urban and forested sites. *Culex quinquefasciatus* (50.17%) and *Ae. aegypti* (38.73%) were the most predominant species in urban areas while *Ae. vittatus* (89%) dominated the forested area among the larval collection. *Ae. tricholabis* (45%) and *Ae. aegypti* (20.0%) were predominant adult species in forested and urban areas with Haller Park recording the highest adult population (87.2%). Out of 638 pools screened for flaviviruses, bunyavirus and alphavirus 23(3.6%) pools were positive for flavivirus and 6(0.9%) for alphavirus by RT-PCR. Haller Park reported highest number of arboviruses with 16(55.2%) flaviviruses and 6(20.7%) alphaviruses followed by Bamburi with 5(17.2%) flaviviruses and Gede with 2(6.9%) flaviviruses. *Ae. aegypti* recorded the highest number of infections rate, with 6(20.7%) in Haller Park, 3(10.3%) in Bamburi and 2(6.9%) in Gede. The two positive pools in Gede were from larval collection. Other positive species in Haller Park included *Ae. tricholabis* (4), *An. Funestus* (3), *Ae. vittatus* (2), *Cx. pipiens* (2), *Cx. vansomereni* (2) and *Ae. hirsutus* and *Fi. Circumtesta* 1 positive sample each. Additionally, flaviviruses were detected in 2 pools of *Ae. vittatus* from Bamburi. Blood fed mosquitoes included *Ae. tricholabis* (10), *Ae. Aegypti* (1) and *Cx. watti* (1). Blood

mel analysis identified various animal hosts, including white tailed mongoose (1), bushbuck (2), *beisa oryx* (1), Somali giraffe (4), and four unidentified sources. The high abundance of *Ae*. *aegypti* in urban, peri-urban and forested areas as well as *Ae*. *tricholabis* in forested areas, poses great risk of arboviruses outbreak in the region due to their role in dengue virus (DENV) transmission. The study confirms active transmission of flaviviruses and alphaviruses in Haller Park, Gede and Bamburi. The high mosquito diversity and population across the study sites combined with their ability to feed on both animals and humans, suggests a potential link between the sylvatic and urban transmission cycles. Further studies, including serological surveys in both humans and animals are recommended to determine the extent of active and past infections. Well-funded longitudinal surveys would be recommended to inform on breeding cycles, seasonal peaks and variations in vector density over time. The key findings of this study will add into the existing knowledge and help establish reliable control and management strategies of arboviral infection to avert future outbreaks.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Arboviruses are arthropod-borne viruses transmitted to humans and animals mainly by mosquitoes. Majority of these infections are self-clearing, though some can be life threatening (Braack et al., 2018; Sang & Dunster, 2001). Many of these important arboviruses are believed to have originated in tropical Africa where, though they are somewhat overshadowed by the high prevalence of malaria, their burden remains significant (LaBeaud, 2008; Mutebi et al., 2004; Powers et al., 2000). Although much of their distribution and epidemiology remains largely unknown, confirmed arboviral outbreaks have impacted heavily on health, agriculture and socio-economic aspects (LaBeaud, A et al., 2011; Woods et al., 2002). During the 1997 and 1998 Rift Valley Fever (RVFV) outbreak in Kenya, 27,500 infection cases were estimated to have occurred, making it the largest RVFV outbreak to have been recorded in East Africa (Woods et al., 2002). Further large RVFV outbreaks occurred in Kenya, Somalia, and Tanzania between late 2006 and 2007, with over 1,060 confirmed human cases and nearly 400 deaths (Munyua et al., 2010; Nderitu et al., 2011; Sumaye et al., 2013). Coastal Kenya has experienced multiple arboviral outbreaks in recent decades. Chikungunya virus (CHIKV) outbreaks occurred in 2003–2005, Rift Valley fever between 2006–2007, and dengue virus (DENV) between 2010 and 2014 in counties such as Kilifi, Mombasa, and Malindi. (Ellis et al., 2015; Lutomiah et al., 2016; Masila et al., 2013; Njenga et al., 2008; Sang et al., 2010) In addition, serological studies conducted at Msambweni and Mombasa over the years from 2000 – 2004 demonstrated significantly high levels of IgG antibodies against Yellow Fever Virus (YFV) and West Nile virus (WNV) (Mease et al., 2011; Sergon et al., 2008; Sutherland et al., 2011). Cases of Zika Virus (ZIKV) have also been reported in Polynesia in 2013 and 2014 (Cao-Lormeau et al., 2014) and Brazil in 2015 (Campos et al., 2015). The known insect vectors that transmit ZIKV are found along the Kenyan Coast, northeastern Kenya, and Rift Valley, among other places, and these vectors include *Ae. aegypti, Ae. africanus, Ae. vittatus, Ae. tarsalis, Ae. furcifer*

and *Eratmapodites* among others (Boyer et al., 2018). Despite the presence of these vectors in Kenya, ZIKV infections have not been reported (Boyer et al., 2018; Campos et al., 2015; Cao-Lormeau et al., 2014).

Transmission of arboviruses majorly involves the sylvatic (jungle) and urban transmission cycles (CDC, 2016c). The sylvatic cycle involves transmission of arboviruses between non-human primates and the forest-based mosquitoes such as *Aedes furcifer Ae. tarsalis, Ae. africanus*, *Ae. vittatus*, whereas the urban transmission cycle is the transmission of viruses between humans and peri-domestic or domestic mosquito species such as *Aedes aegypti (Boyer et al., 2018; CDC, 2016)*. The communities bordering the forested areas could act as the link between these two cycles. The community members acquire the arboviral infections found circulating in the forest when they go for hunting or to gather firewood or fruits (CDC, 2016). Thereafter, the peri-domestic and domestic insect vectors may acquire the bloodmeals from the infected individuals, and therefore further spreading infections across the rural, peri urban and urban regions (CDC, 2016).

Arboviral transmission within the urban regions intensifies during the dry seasons when people tend to preserve water in containers within their houses, thus providing ample breeding habitats for the main arboviral-vector, *Aedes aegypti* (Angel & Joshi, 2008; Chretien et al., 2007). Notably, Chikungunya and dengue fever outbreaks have been reported by several studies in relation to increased usage of domestic water storage containers during drought periods (Angel & Joshi, 2008; Chretien et al., 2007). Also, during short rainy seasons or when the long rains are subsiding, vector densities increase rapidly due to increased stable breeding sites, which leads to arboviral outbreaks (Angel & Joshi, 2008). Thus, the water collects and stays long enough to allow eggs to hatch and develop into adult mosquitoes. These breeding sites include: discarded containers, old boats, coconut husks, poorly drained areas, rock holes, plant axils and tires (Angel & Joshi, 2008; Nguku et al., 2010). Apart from arbovirus spillover from the sylvatic cycle, outbreaks in the urban regions could be due to importation of infections from other regions with active virus transmission (Nguku et al., 2010). Similarly, rainy seasons have been associated with increased transmission of arboviruses in forest due to high densities of sylvatic *Aedes*

mosquitoes (Angel & Joshi, 2008). Trans-ovarian/vertical and venereal transmission also plays a critical role in maintaining active transmission of the viruses in both urban and forest regions (Clements, 2012). An entomological based study carried out in Senegal during 1995 YFV outbreak reported the first evidence of vertical transmission in nature (Fontenille et al., 1997). Similarly, several laboratory-based studies have also reported vertical and venereal transmission of arboviruses (Agarwal et al., 2014; M. Diallo et al., 2000; Mavale et al., 2010). The reservoirs such as monkeys, galago baboo and kangaroos could also play important role in maintenance of arboviruses in the sylvatic transmission (Clements, 2012).

1.2 Statement of the Problem

Arboviral diseases constitute a major public health, veterinary, and economic challenge in Kenya, with recurrent outbreaks of dengue, chikungunya, yellow fever, and Rift Valley fever reported particularly in the Coastal, northeastern, and Rift Valley regions (Ellis et al., 2015; Masila et al., 2013; Sergon et al., 2008). These infections cause a wide range of outcomes in humans, from mild febrile illness to severe hemorrhagic disease, neurological complications, and death, placing substantial strain on healthcare systems and contributing to loss of productivity and household income (Hertz et al., 2012; LaBeaud, A et al., 2011; Nigussie et al., 2024). In animals, especially livestock, arboviruses such as Rift Valley fever virus result in high mortality, abortion storms, and reduced productivity, leading to significant financial losses and disruption of livestock trade, food security, and national economic stability (Njenga et al., 2008; Sang et al., 2010). Despite this burden, the ecological drivers sustaining arbovirus transmission remain poorly understood, particularly the role of sylvatic transmission cycles and mosquito host-feeding behavior in forested ecosystems bordering coastal urban areas. Overlapping clinical symptoms with malaria further contribute to misdiagnosis and under-recognition of outbreaks, underscoring the need for improved surveillance tools (Hertz et al., 2012). Molecular and bioinformatic approaches, including DNA-based blood-meal analysis, sequence alignment, database comparison, and phylogenetic inference, offer powerful means to identify vertebrate hosts, characterize vector–host interactions, and elucidate transmission pathways that maintain arboviruses during inter-epidemic

periods (Alcaide et al., 2009; Kumar et al., 2018)**.** Without such integrated molecular and bioinformatic evidence, current control strategies remain reactive, limiting their effectiveness in preventing spillover from sylvatic to human transmission cycles in Kenya's rapidly changing landscapes (Eastwood et al., 2020)**.**

1.3 Justification

Understanding how arboviruses persist in nature; particularly the role of sylvatic transmission cycles during inter-epidemic periods; is essential for accurately characterizing their local epidemiology and anticipating future risks to human and animal populations. Substantial evidence indicates that vertical (transovarial) transmission in mosquitoes contributes to long-term virus maintenance, especially during unfavourable climatic conditions such as dry seasons. In Kenya's Lake Victoria and Lake Baringo regions, arboviruses have been detected across multiple mosquito generations reared from field-collected larvae, supporting the role of vertical transmission in the persistence of bunyaviruses and flaviviruses in natural ecosystems (Ajamma et al., 2018; Auguste et al., 2010; M. Diallo et al., 2000; Eastwood et al., 2020; Joshi et al., 2002; Mavale et al., 2010; Murillo, Holechek, & Murillo, 2014).

Historical evidence further supports the existence of sylvatic transmission cycles in East Africa. Kenya's first documented yellow fever outbreak in 1992–1993 occurred in the Kerio Valley, where virus isolation from *Aedes africanus* and *Aedes keniensis* demonstrated a classic sylvatic transmission pattern independent of urban cycles (Abdulwahab Alhakimi et al., 2015; Diawo. Diallo et al., 2012; Reiter et al., 1998; Sanders et al., 1998). Although direct evidence of continuous sylvatic maintenance systems remains limited in East Africa, the lack of correlation between entomological indices; typically associated with urban transmission; and attack or fatality rates during the 2012 Darfur yellow fever outbreak suggests the involvement of sylvatic transmission prior to and during the epidemic (Abdulwahab Alhakimi et al., 2015; Uwishema et al., 2022).

While viruses such as yellow fever virus (YFV), chikungunya virus (CHIKV), Zika virus (ZIKV), and dengue virus (DENV) possess the theoretical capacity for vertical

transmission, outbreak emergence is more likely driven by zoonotic spillover from sylvatic reservoirs or the introduction of infected humans, rather than sustained urban transmission in isolation (Agha et al., 2022; Uwishema et al., 2022). This study therefore seeks to elucidate arbovirus transmission dynamics at coastal Kenyan forest–urban interfaces by examining sylvatic and urban mosquito populations, assessing evidence of vertical transmission, and identifying potential links between forest-based and human transmission cycles. The findings are expected to generate critical evidence to support locally tailored vector control, surveillance, and disease prevention strategies grounded in ecological and entomological realities.

1.4 Objectives

1.4.1 Main objective

To establish the transmission dynamics of arboviruses along the coastal Kenya

1.4.2 Specific objectives

(i) To determine the relative abundance and distribution of the mosquito species associated with arbovirus transmission
(ii) To assess the current potential vectors of arboviruses transmission in the study areas
(iii) To determine the link between the sylvatic and urban transmission cycles
(iv) To determine the host preference for the blood fed mosquitoes sampled from study area.

1.5 Research Questions

(i) What is the relative abundance and distribution of arbovirus vectors in the study sites
(ii) What is the range of potential vectors for arboviruses among the mosquitoes present in the Kenyan coastal region?
(iii) What is the relationship between the sylvatic and urban transmission cycles of arboviruses along the Kenyan coast?

(iv) Which arboviruses are harbored by local vector populations, and what are the host preferences of blood-fed mosquitoes?

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Arboviruses (arthropod-borne viruses) are a diverse group of viruses transmitted to humans and animals primarily through the bites of infected mosquitoes, ticks, and other arthropods. Globally, arboviral diseases such as dengue, chikungunya, yellow fever, and Rift Valley fever pose significant public health and socio-economic threats, particularly in tropical and subtropical regions. These infections range from mild febrile illnesses to severe manifestations such as hemorrhagic fevers and encephalitis (Lequime & Lambrechts, 2014; Ochieng et al., 2015).

Kenya, especially the coastal region, has been identified as a hotspot for arboviral outbreaks due to its favorable climatic conditions, high vector diversity, and rapid urbanization. Historical outbreaks of Rift Valley fever (1997–1998, 2006–2007), dengue virus (2011, 2013–2014), and chikungunya virus (2004, 2018) have caused considerable morbidity and economic losses (Chretien et al., 2007; Ellis et al., 2015; Lutomiah et al., 2016; Munyua et al., 2010; Sang et al., 2010; Sergon et al., 2008; Woods et al., 2002). These outbreaks highlight the complexity of arboviral transmission dynamics, which are shaped by interactions between mosquito vectors, animal reservoirs, human populations, and environmental factors.

A key feature of arbovirus ecology in coastal Kenya is the co-existence of urban and sylvatic transmission cycles**,** often interconnected through bridge vectors such as *Aedes aegypti* and *Aedes vittatus*. The sylvatic cycle; where arboviruses circulate between wild animals and forest-dwelling mosquitoes; remains poorly understood in Kenya, yet it may play a critical role in virus maintenance during inter-epidemic periods. Environmental drivers, such as increased rainfall, deforestation, and urban expansion, create ideal breeding habitats for mosquitoes, increasing the risk of spillover events. Additionally, the overlapping clinical symptoms of arboviral infections with malaria complicate diagnosis and reporting (Hertz et al., 2012). Understanding the transmission dynamics of arboviruses in coastal Kenya is vital for

developing early-warning surveillance systems, designing effective vector control strategies, and reducing the risk of future outbreaks.

2.2 Arboviruses of Public Health Importance in Kenya

Kenya has experienced multiple arboviral outbreaks over the past decades, with the coastal, northeastern, and Rift Valley regions being the most affected. Several arboviruses of medical and veterinary significance have been documented, including Dengue virus (DENV), Chikungunya virus (CHIKV), Rift Valley fever virus (RVFV), Yellow fever virus (YFV), and Zika virus (ZIKV), among others (Ellis et al., 2015; Lutomiah et al., 2016; Sang et al., 2010).

2.2.1 Dengue Virus (DENV)

Dengue virus has emerged as a major public health concern in Kenya, particularly in Mombasa, Malindi, and Kilifi counties. DENV outbreaks have been recorded in 2011, 2013–2014, and 2019, with a predominance of serotypes DENV-1 and DENV-2 (Konongoi et al., 2016; Mwanyika et al., 2021). The presence of Aedes aegypti, a highly efficient vector of DENV, has been a key driver of these outbreaks.

2.2.2 Chikungunya Virus (CHIKV)

Chikungunya virus has also caused significant outbreaks in coastal Kenya, with the 2004–2005 epidemic spreading rapidly due to high vector densities and favourable climatic conditions (Sergon et al., 2008). The re-emergence of CHIKV in 2018 further underscores its persistent threat (East, 2018; Lutomiah et al., 2021; WHO, 2018)

2.2.3 Rift Valley Fever Virus (RVFV)

Rift Valley fever virus is endemic in Kenya, with major outbreaks recorded in 1997–1998 and 2006–2007. These outbreaks not only caused human morbidity and mortality but also resulted in significant economic losses in the livestock sector due to trade restrictions and animal deaths (Munyua et al., 2010; Sang et al., 2010; Woods et al., 2002)

2.2.4 Yellow Fever Virus (YFV)

Yellow fever virus outbreaks, although infrequent, have been reported historically in Rift Valley and northern Kenya. Surveillance data indicate that potential YFV vectors, such as *Aedes bromeliae* and *Ae. africanus*, are present in coastal areas (Agha et al., 2017, 2022).

2.2.5 Zika Virus (ZIKV)

Although **Zika virus (ZIKV)** has not yet caused reported outbreaks in Kenya, the presence of competent vectors (*Ae. aegypti*, *Ae. vittatus*) suggests a potential risk for future spillover events (Boyer et al., 2018)

2.3 Epidemiology of Arboviruses in Coastal Kenya

The coastal region of Kenya, comprising counties such as Mombasa, Kilifi, Lamu, Kwale, and Tana River, has emerged as a hotspot for arboviral outbreaks due to its tropical climate, high mosquito density, and rapid urbanization. Over the past two decades, multiple arboviruses; including dengue virus (DENV), chikungunya virus (CHIKV), and Rift Valley fever virus (RVFV); have been documented in the region, often occurring in sporadic but intense outbreaks (Lutomiah et al., 2016, 2021).

2.3.1 Dengue Virus

Dengue virus (DENV) has been the most frequently reported arbovirus along the Kenyan coast. The first confirmed DENV outbreak occurred in 1982, but since 2011, outbreaks have been increasingly reported, with major epidemics recorded in Mombasa and Malindi during 2013–2014 and again in 2017–2019 (Konongoi et al., 2016; Mwanyika et al., 2021). Seroprevalence studies reveal that a significant proportion of the population in coastal counties has antibodies against DENV, indicating both past exposure and ongoing transmission (Mease et al., 2011; Ochieng et al., 2015).

2.3.2 Chikungunya Virus (CHIKV)

Chikungunya virus has also caused repeated outbreaks in coastal Kenya. The 2004–2005 epidemic spread rapidly across Lamu and Mombasa due to high densities of *Aedes aegypti* and *Ae. vittatus* mosquitoes, combined with favorable climatic conditions (Sergon et al., 2008). The re-emergence of CHIKV in 2018 highlights its persistence and the potential for future outbreaks (Lutomiah et al., 2021)

2.3.3 Rift Valley Fever Virus (RVFV)

Rift Valley fever virus although primarily associated with livestock, has occasionally spilled over into human populations along the coastal and northeastern regions. The 2006–2007 outbreak was particularly severe, resulting in hundreds of human cases and significant livestock losses (Munyua et al., 2010; Nguku et al., 2010).

2.4 Diagnosis, Treatment and Prevention of Arboviruses

2.4.1 Diagnosis of Arboviral Infections

Accurate diagnosis of arboviral infections is essential for patient management, outbreak detection, and surveillance. Diagnostic approaches depend largely on the timing of sample collection relative to symptom onset. During the acute phase of infection, molecular methods such as reverse transcription polymerase chain reaction (RT-PCR) are considered the gold standard, as they enable direct detection of viral RNA with high sensitivity and specificity (Weaver & Reisen, 2010). For dengue virus, antigen-based assays targeting the non-structural protein 1 (NS1) are widely used for early diagnosis, particularly in resource-limited settings.

Serological assays detecting virus-specific IgM and IgG antibodies are commonly used after the acute phase. However, interpretation of serological results is complicated by cross-reactivity among antigenically related arboviruses, particularly flaviviruses such as dengue, yellow fever, and Zika viruses (Guzmán & Kourí, 2004). Plaque reduction neutralization tests (PRNT) remain the reference standard for confirming infection but are costly and technically demanding.

2.4.2 Treatment and Clinical Management of Arboviruses

Currently, no specific antiviral therapies are approved for most arboviral infections, and treatment remains largely supportive. Management of dengue virus infection focuses on careful clinical monitoring and judicious fluid management to prevent complications such as shock and haemorrhage (Martinez et al., 2015; Weaver & Reisen, 2010). Chikungunya virus infection is treated symptomatically using analgesics and anti-inflammatory drugs, although a subset of patients may develop chronic inflammatory arthritis requiring long-term management (Simon et al., 2011).

For Rift Valley fever virus infection, supportive care remains the primary treatment approach, particularly for severe manifestations such as haemorrhagic fever or encephalitis. Although antiviral agents such as ribavirin and favipiravir have shown efficacy in experimental studies, their clinical use in humans remains limited (Bird & Nichol, 2012).Overall, early diagnosis and prompt supportive care are critical in reducing morbidity and mortality associated with arboviral diseases.

2.4.3 Prevention and Control of Arboviruses

Prevention of arboviral diseases is built on evidence-based vector control strategies aimed at reducing mosquito populations and human–vector contact. Integrated Aedes Management (IAM) combines vector surveillance, environmental management, targeted larviciding, and community mobilization to sustainably suppress populations of Aedes mosquitoes and lower arbovirus transmission risk (Roiz et al., 2018). Field studies demonstrate that integrated household-level control strategies can reduce local mosquito densities, while complementary approaches such as the sterile insect technique further augment vector suppression efforts (Melo et al., 2024; Parrondo Monton et al., 2025). Community education and active participation in breeding-site elimination have also been shown to improve preventive practices and decrease entomological risk indicators (Roiz et al., 2018). Recent evidence from Africa highlights the need for tailored, evidence-driven control plans that integrate larval control, environmental management, and community engagement as part of routine and outbreak response strategies (Roiz et al., 2018).

2.5 Mosquito Vector Ecology and Population Dynamics

Mosquito vectors play a pivotal role in the transmission of arboviruses along the Kenyan coast, with *Aedes aegypti* and *Aedes vittatus* being the primary species associated with urban and peri-urban transmission. Other important species include *Culex quinquefasciatus*, *Anopheles funestus*, and forest-associated vectors such as *Aedes africanus* and *Aedes bromeliae*, which contribute to the sylvatic cycle (Karuitha et al., 2019; Lutomiah et al., 2016, 2021).

2.5.1 Vector Diversity and Distribution

The coastal region's warm, humid climate and extensive vegetation create ideal breeding conditions for a wide diversity of mosquitoes. Urban and peri-urban areas provide artificial breeding habitats such as discarded tires, water containers, and blocked drains, which favor the proliferation of *Ae. aegypti*, the primary vector of dengue, chikungunya, and Zika viruses (Ellis et al., 2015; Karuitha et al., 2019; Lutomiah et al., 2016). In contrast, forested regions support sylvatic vectors like *Ae. vittatus* and *Ae. africanus*, which act as bridge vectors between wildlife reservoirs and humans (Eastwood et al., 2020; Karuitha et al., 2019)

2.5.2 Seasonal Population Dynamics

Mosquito abundance in coastal Kenya shows significant seasonal variation, with population peaks observed during the rainy seasons (April–June and October–December). Rainfall creates temporary pools and expands breeding habitats, while elevated humidity increases vector survival and biting rates (Ngugi et al., 2017). These seasonal changes are closely linked to spikes in arboviral transmission.

2.5.3 Vector Competence and Adaptation

Studies have demonstrated the high vectorial capacity of *Ae. aegypti* populations in coastal Kenya, particularly for DENV and CHIKV, while *Ae. bromeliae* and *Ae. africanus* are considered efficient vectors of yellow fever virus in sylvatic settings (Eastwood et al., 2020; Karuitha et al., 2019; Lutomiah et al., 2016). Moreover, rapid

urbanization and climate variability are enhancing the adaptation of mosquitoes to peri-domestic environments, increasing human-vector contact.

2.5.4 Insecticide Resistance

While specific Kenyan data are scarce, pyrethroid resistance in *Aedes aegypt* have been reported in Tanzania, posing a challenge for vector control strategies (Emidi et al., 2024). No dedicated peer-reviewed bioassay study has been published solely on *Aedes aegypti* insecticide resistance in Kenya, despite widely use of pyrethroid treated mosquito nets, highlighting a research gap.

2.6 Transmission Cycles of Arboviruses

The transmission dynamics of arboviruses along the Kenyan coast are influenced by a combination of urban, sylvatic, and vertical (transovarial) cycles, which together sustain viral persistence and facilitate spillover into human populations. Figure 1.1 bellow shows transmission cycle of yellow fever.

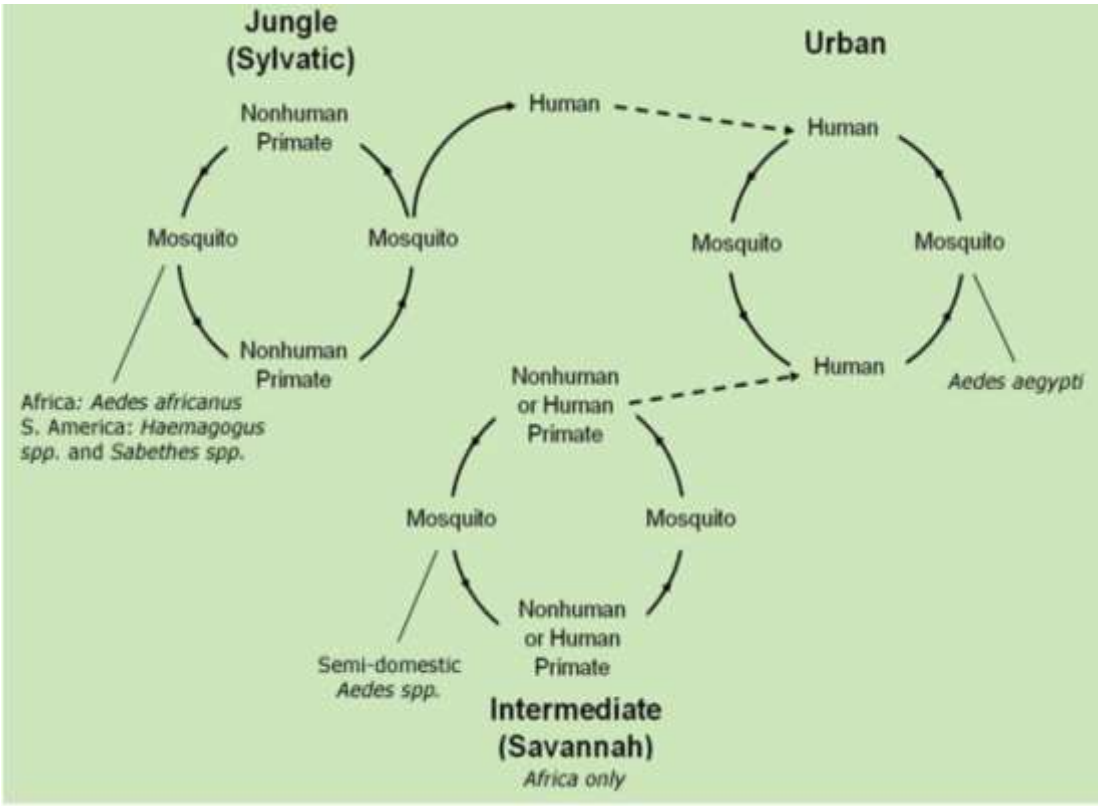

Figure 2.1: Yellow Fever Transmission Cycle; and Arbovirus Transmission Cycle Involving the Three Transmission Cycles;

The sylvatic transmission cycle involve transmission of YFV between non-human primates such as monkeys and mosquitoes, primarily *Aedes* species. The virus is transmitted from monkeys to human working in the forest or living nearby forests through infected mosquito bite. The intermediate cycle involve transmission of YFV from monkey to human or human-to-human through tree hole-breeding *Aedes* mosquito species. The urban transmission cycle involve transmission of YFV between humans and *Ae.aegypti* or *Ae.albopictus*. Source:(Gershman & Staples, 2016).

2.6.1 Urban Transmission Cycle

In urban settings, arboviruses such as dengue virus (DENV), chikungunya virus (CHIKV), and Zika virus (ZIKV) are primarily transmitted between humans and *Aedes aegypti*, the dominant urban vector. Poor waste management, high human density, and stagnant water containers (e.g., tires and tanks) create ideal breeding conditions for *Ae. aegypti*. The close association of this species with humans and its multiple feeding behavior (biting several hosts in one gonotrophic cycle) make it a highly efficient vector for urban transmission (Ellis et al., 2015; Karuitha et al., 2019; Lutomiah et al., 2016; Mwanyika et al., 2021).

2.6.2 Sylvatic Transmission Cycle

The sylvatic (forest-based) cycle involves arbovirus maintenance between wild animals such as monkeys, antelopes, and rodents and forest-dwelling mosquito species like *Aedes africanus*, *Aedes vittatus*, and *Aedes bromeliae* (Eastwood et al., 2020; Gershman & Staples, 2016; Karuitha et al., 2019; Thiboutot et al., 2010). Coastal forests, such as Arabuko-Sokoke, provide habitats where these vectors thrive. Spillover occurs when humans encroach into forested areas for logging, hunting, or agriculture, increasing contact with infected vectors (Eastwood et al., 2020). Sylvatic cycles are particularly important for yellow fever virus (YFV) and Rift Valley fever virus (RVFV), serving as reservoirs during inter-epidemic periods (Gershman & Staples, 2016).

2.6.3 Vertical (Transovarial) Transmission

Vertical transmission, where arboviruses are passed from infected female mosquitoes to their eggs, allows viruses to persist in vector populations during adverse conditions, such as the dry season Evidence of transovarial transmission has been reported for DENV, YFV, and CHIKV, suggesting that arboviruses can survive inter-epidemic periods even in the absence of vertebrate hosts (Diallo M et al., 2000; Fontenille et al., 1997; Murillo, Holechek, & Murillo, 2014).

2.6.4 Intermediate Cycles and Spillover

The interaction of sylvatic and urban cycles, often through “bridge vectors” like *Ae. vittatus*, facilitates rapid spread of viruses across rural and urban settings. Such dynamics were evident in past CHIKV and DENV outbreaks along the coastal belt (Gershman & Staples, 2016; Thiboutot et al., 2010)

2.7 Host Preferences and Blood-Feeding Behavior

Host selection and feeding patterns of mosquito vectors are central to the transmission dynamics of arboviruses. Different mosquito species show varying preferences for humans or animals, influencing the risk of zoonotic spillover and urban outbreaks.

2.7.1 Human- Biting Preference

Aedes aegypti, the dominant urban vector in coastal Kenya, is highly anthropophilic (human-preferring) and frequently bites multiple individuals during a single feeding cycle, significantly increasing the chances of virus transmission. Its close association with human dwellings, coupled with daytime biting behaviour, enhances the efficiency of dengue, chikungunya, and Zika virus transmission (Kamau et al., 2023).

2.7.2 Animal Reservoirs and Sylvatic Vectors

In contrast, forest-dwelling mosquitoes such as *Aedes vittatus*, *Aedes africanus*, and *Aedes furcifer* feed on a wide range of wild animals, including non-human primates, antelopes, and rodents, which serve as important reservoirs of arboviruses like yellow fever virus (YFV) and Rift Valley fever virus (RVFV)(Diawo. Diallo et al., 2012). These vectors act as "bridge species," transmitting pathogens from sylvatic cycles to humans when forest habitats are disturbed or when humans engage in activities near forested regions.

2.8 Molecular and Bioinformatic Approaches

Advances in molecular biology and bioinformatics have significantly enhanced the study of vector–host interactions by enabling accurate identification of blood-meal sources and genetic characterization of biological samples. Molecular techniques such as DNA extraction, polymerase chain reaction (PCR), and Sanger sequencing allow for the amplification and analysis of conserved genetic markers, including mitochondrial genes, which are particularly useful for species identification due to their high copy number and interspecific variability (Alcaide et al., 2009; Hebert et al., 2003). These approaches provide greater sensitivity and specificity compared to traditional serological or morphological methods, especially when working with small or partially degraded blood-meal samples.

Bioinformatic analyses complement molecular methods by enabling sequence quality assessment, multiple sequence alignment, database comparison, and phylogenetic inference. Sequence similarity searches using the Basic Local Alignment Search

Tool (BLAST) against public repositories such as GenBank facilitate reliable taxonomic assignment of unknown sequences (Benson et al., 2014; Boratyn et al., 2013). Multiple sequence alignment tools, such as MAFFT, improve alignment accuracy and computational efficiency for comparative analyses (Katoh et al., 2018), while phylogenetic reconstruction software such as MEGA allows for the visualization of evolutionary relationships and validation of species identification through bootstrap-supported clustering (Kumar et al., 2018; Tamura et al., 2013). Together, molecular and bioinformatic approaches provide a robust and reproducible framework for investigating host-feeding patterns, host diversity, and potential pathways for pathogen transmission, thereby contributing to a deeper understanding of vector-borne disease ecology and epidemiology.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site Description and Site Selection

The study was conducted in Mombasa and Kilifi counties along the Kenyan coast, focusing on urban, peri-urban, and forested ecosystems with known or suspected arbovirus activity. Mombasa Island (4°00′ S, 39°04′ E), the commercial and administrative hub of the region, and its adjacent mainland areas; Likoni, Changamwe, Nyali, and Bamburi; were surveyed for mosquito breeding sites in 2014. These areas are characterized by dense human populations, mixed residential and commercial land use, and the presence of artificial breeding habitats such as water storage containers and discarded tires.

Key ecological sites included Haller Park (4°01′00″ S, 39°43′10″ E), a rehabilitated limestone quarry north of Mombasa city, now a nature reserve hosting diverse wildlife, including giraffes, buffalo, hippos, antelopes, monkeys, and over 160 bird species (Haller, 2023a, 2023b). Bamburi (4°00′ S, 39°43′ E), a peri-urban area nearby, combines residential settlements, tourist attractions, and industrial activities. Nyali (4°03′00″ S, 39°42′00″ E) is a high-income residential area connected to Mombasa Island via Nyali Bridge, while Likoni (4°05′00″ S, 39°39′00″ E) is a densely populated mainland area accessible only by ferry. Changamwe (4°01′34″ S, 39°37′50″ E) is an industrial zone west of Mombasa Island.

In Kilifi County, Arabuko-Sokoke Forest (3°35′81″ S, 30°89′90″ E) was a primary forested study site. It is the largest intact coastal forest in East Africa, covering approximately 370 km², and is home to elephants, yellow baboons, lesser galagos, and numerous bird species (Matiku, 2002). Gede (3°28′00″ S, 39°18′00″ E), a peri-urban area along the Mombasa–Malindi highway, was included due to its proximity to Arabuko-Sokoke and presence of tourist attractions such as Gede Ruins as shown in Figure 3.1.

The region experiences a tropical climate with bimodal rainfall; long rains (April–June) and short rains (November–December); with an annual precipitation of ~1,192 mm, an average temperature of 24.7°C, and 80% humidity. Surveys conducted in 2014 and 2016 focused on mosquito larval habitats, adult species diversity, and egg collections, targeting areas linked to previous dengue outbreaks(Karuitha et al., 2019; Lutomiah et al., 2016).

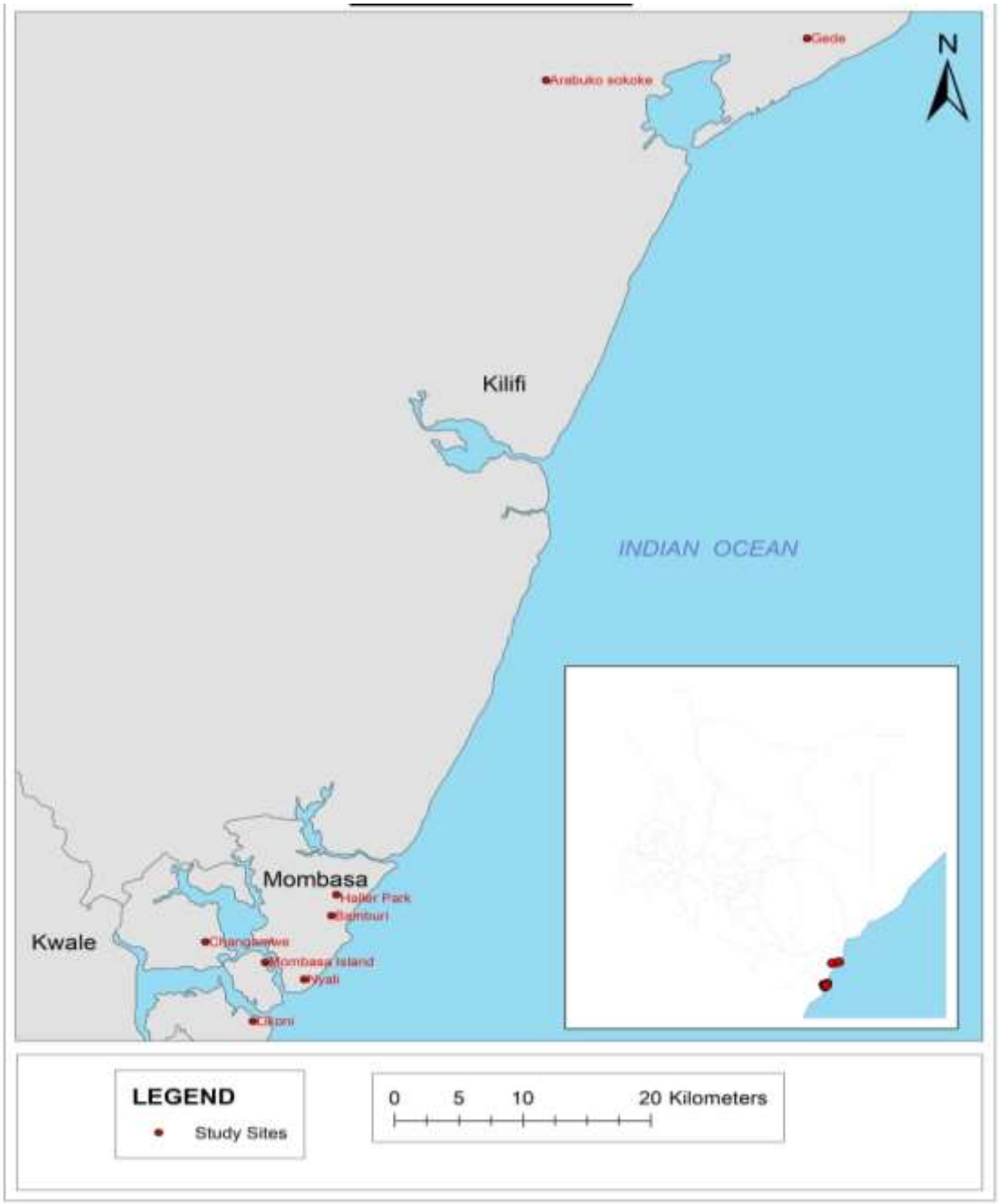

Figure 3.1: Map of Coastal Kenya Showing Mombasa and Kilifi Counties, with the Selected Study Locations for Arbovirus Surveillance:

Urban sites (Mombasa Island, Nyali, Likoni, Changamwe), peri-urban zones (Bamburi, Gede), and the forested area (Arabuko-Sokoke Forest) are identified. Coordinates are annotated for each site.

3.2 Mosquito Collection and Laboratory Processing

3.2.1 Study Design

This study employed a cross-sectional entomological survey to assess the distribution and diversity of mosquito populations and other transmission dynamics of arboviruses across the selected ecological zones along the Kenyan coast. The survey was conducted during a single rainy season from the selected eight (8) sites as indicated in Figure 3.1 above

3.2.2 Sample Size

Mosquito eggs, larvae, and adult mosquitoes were systematically collected to provide a comprehensive snapshot of vector presence and diversity. Larval sampling involved thorough inspection of all potential breeding habitats both artificial and natural. All positive larval habitats, defined as any container or water-holding site with observable mosquito larvae, were sampled. The sampling was done as described in sub-headings below.

3.2.3 Collection of Mosquito Eggs

A total of thirty ovicups were placed in randomly selected locations (on tree holes, rock holes, between branches, under shrubs and between bamboo trees) in Haller Park (n = 15) and Arabuko Sokoke forest (n = 15) targeting *Aedes* mosquitoes and retracted 5 days later (Figure 3.2). The filter paper lining the ovicup was air dried, packed in individually labelled envelopes and transported to Biosafety level-2 insectary at Kenya Medical Research Institute-Centre for Virus research (KEMRI-CVR). The dried eggs were then dispensed in larval layering trays to hatch and the larval reared to adults under controlled laboratory conditions as shown in Figure 3.3. The emerging adults were knocked down at 4^{o} C and preserved at -80^{o} C in 1.5ml cryogenic tube for further processing.

Figure 3.2: Ovitrap Cup Placed in a Shaded Vegetated Area and Under the Rocks for the Collection of Aedes Mosquito Eggs

*The container, partially concealed by leaves and natural debris, mimics natural breeding habitats to attract ovipositing female mosquitoes.

Figure 3.3: Mosquito Eggs Dispensation and Larvae Rearing in Insectary

*The white trays are labelled and arranged in the order by collection dates and site from the top

3.2.4 Larval Habitat Identification and Sampling

All the study areas were surveyed for mosquito breeding habitats which were sampled for mosquito larvae. For consistency, sampling was done concurrently in Mombasa Island, Likoni, Changamwe and Kisauni sub-counties in Mombasa with guidance from the local public health officers (PHOs) and local guides in Haller

Park, Bamburi, Gede and Arabuko Sokoke forest. Each breeding habitat was georeferenced using hand-held wireless global positioning systems (GPS). Mosquito larval sampling was conducted between August and October 2014 in Mombasa Island and its environs and from November to December 2016 in Haller Park, Arabuko Sokoke forest, Gede and Bamburi. Depending on the habitat size, mosquito larvae were sampled using either standard dipping (A) or pipetting techniques (B) (Figures 3.4). One to three dipper samples were taken along the habitat edge depending on the habitat size using a 350 ml dipper (Mwangangi et al., 2007; Njoroge et al., 2015; Service, 1993). In small habitats where 350 ml dipper could not be used or contained less than half a litter of water, 1ml transfer pipette was used to collect mosquito larvae and pupae. The samples for each habitat on each sampling occasion were enumerated, pooled into larval bottles and transferred to the laboratory in a cool box for rearing, identification and further processing.

Figure 3.4: Standard Dipper Used for Sampling Mosquito Larvae from Natural and Artificial Water Habitats During Field Surveillance

*This method allows for rapid assessment of larval presence and habitat productivity. Image A: a standard 350ml dipper for scoping larvae in medium and large habitats. Image B: Pipetting technique applied for collecting individual mosquito larvae from sampling containers into vials for identification and laboratory rearingg.

3.2.5 Adult Mosquito Collection and Transportation

Adult mosquitoes were collected from two study sites in Mombasa County (Haller Park and Bamburi), and two in Kilifi County (Arabuko Sokoke forest and Gede) between November and December 2016. Three adult mosquitos' collection methods were including Center for Disease Control and Prevention (CDC) resting trap (A), CO2-baited CDC light traps (B) and Biogents (BG) Sentinel trap (C) (Figure 3.5). In each of the sampling locations, ten sets of BG and Light traps and five sets of resting traps were set randomly at different points within the same study area at 1800 hours, away from animal path and collected between 0600 and 0800 hours the following day. Another set of traps were placed at different locations within the same site at 0600 hours, targeting diurnal feeding mosquitoes and retrieved at 1800 hours. Trapped mosquitoes were knocked down using Triethylamine Acetate (TEA), then sorted to remove non-targeted insects, and preserved in liquid nitrogen shippers for transportation to KEMRI-Centre for Virus Research in Nairobi for identification and further processing.

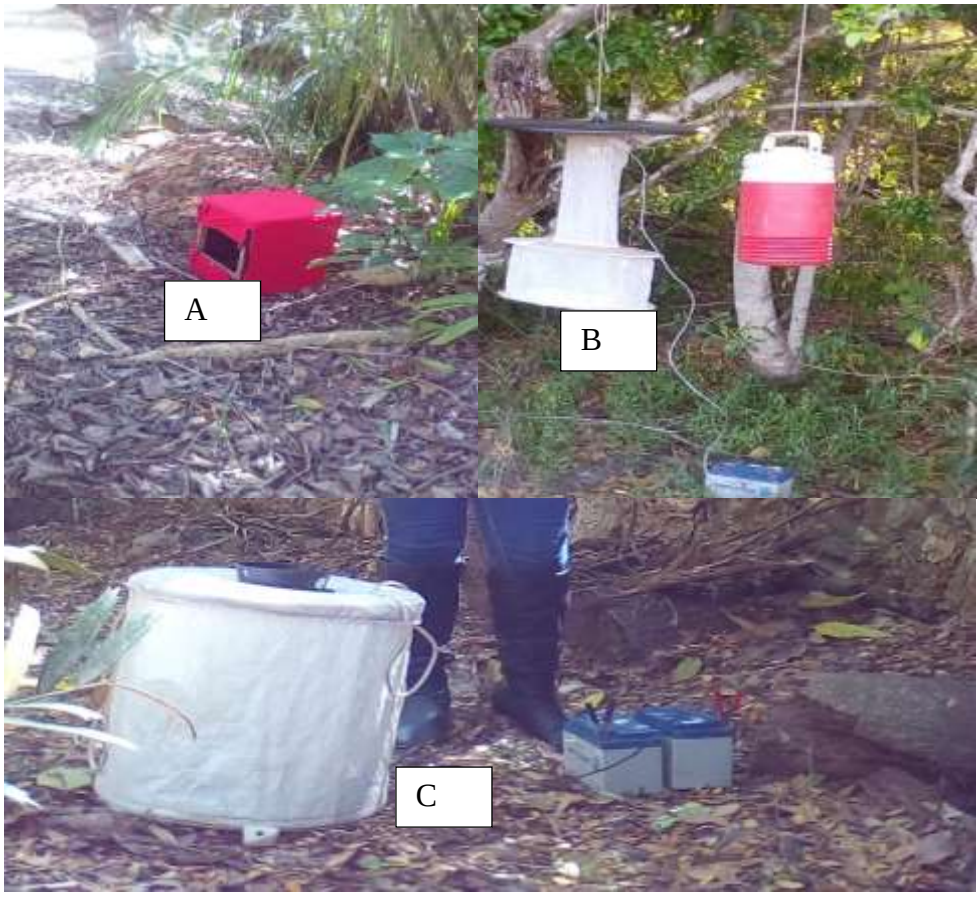

Figure 3.5: Adult Mosquito Collection Traps Used During Field Surveillance

*(A) CDC Resting Trap for sampling resting mosquitoes from vegetation and shelters; (B) CDC Light Trap, typically used overnight to attract host-seeking mosquitoes using light cues; and (C) BG-Sentinel Trap, designed to attract Aedes species using human scent lures and visual cues. These complementary tools enhance mosquito species diversity and abundance data for vector surveillance."

3.3 Laboratory Processing

3.3.1 Adult Mosquito and Adult Mosquito Identification

The larvae from each aquatic habitat were transferred into white-enamel trays for rearing at the insectary. The date of collection, habitat type, and site were labelled. The pupae were placed in pupae cages and reared to adults under laboratory conditions. Adult mosquitoes were identified morphologically over ice block to species using identification keys described by (Gillet, 1972; Gillies & Coetzee, 1987;

Harbach, 1988; Jupp et al., 1980), and pooled into groups of up to 25 mosquitoes each in 1.5 ml Eppendorf tube according to; species, sex, site, and collection date giving 638 pools which were preserved at -80 ^{o} C freezers for future processing.

3.3.2 Cell Culture

3.3.2.1 Cell Lines

Two cell lines, Vero (obtained from green monkey kidney) and C6/36 (from *Ae. Albopictus* were used in this study following inhouse standard operating protocol (SOP). Both Vero and C6/36 cells were amplified in growth medium (GM) and maintained in maintenance medium (MM) in T75 culture flasks. Vero cells were then incubated at 37 ^{o} C in humidified incubator with 5.0% CO_2, while the C6/36 cells were incubated at 28^{o} C in a humidified incubator with 5.0% CO_2.

3.3.2.2 Homogenizing Media (HM)

HM was prepared by transferring 4.7gm of minimum essential medium powder into a sterile bottle and dissolving in 500 ml double distilled water then sterilizing the medium at 120^{o} C for 15 minutes. The sterile medium was the supplemented with 15% heat inactivated FBS, 2% l-glutamine, 0.4% none essential medium, 6ml of 7.5% sodium bicarbonate and 2% antibiotic/antimycotic solution. One ml of the prepared media was added into 4ml of sterile thioglycolate media for quality control testing and observed for five days post preparation for growth of any microorganism or turbidity before use as per VHF standard operating protocol (SOP).

3.3.2.3 Growth Media (GM)

GM was prepared by transferring 4.7 gm of minimum essential medium powder into a sterile bottle and dissolving in 500 ml double distilled water then sterilizing the medium at 120^{o} C for 15 minutes. The sterile medium was the supplemented with 10% heat inactivated FBS, 2% l-glutamine, 0.4% none essential medium, 6ml of 7.5% sodium bicarbonate and 2% antibiotic/antimycotic solution. One ml of the prepared media was added into 3ml of sterile thioglycolate media for quality control

testing and observed for five days post preparation for growth of any microorganism or turbidity before use as per inhouse SOP.

3.3.2.4 Maintenance Media (MM)

MM was prepared by transferring 4.7gm of minimum essential medium powder into a sterile bottle and dissolving in 500 ml double distilled water then sterilizing the medium at 120^{o} C for 15 minutes. The sterile medium was the supplemented with 2% heat inactivated FBS, 2% l-glutamine, 0.4% none essential medium, 6ml of 7.5% sodium bicarbonate and 2% antibiotic/antimycotic solution. One ml of the prepared media was added into 3ml of sterile thioglycolate media for quality control testing and observed for five days post preparation for growth of any microorganism or turbidity following the inhouse SOP.

3.3.2.5 1X Phosphate Buffer Saline (PBS)

1X PBS was prepared by dissolving 5 PBS tablet in 1000 ml double distilled water in a sterile bottle and then sterilized at 120^{o} C for 15 minutes

3.3.2.6 Virus Isolation by Cell Culture

By manual crushing, the 638 mosquito pools were homogenized in 1.5 ml Eppendorf tubes containing 4.5 mm diameter copper bead and 1 ml homogenizing medium. Homogenate was then clarified by centrifugation at 12000 rpm for 10 minutes at 4^{o} C and the supernatants aliquots stored at -80^{o} C in 1.5 mL Cryovials. Fifty microliters (µl) of the supernatant from each mosquito pool were then inoculated into single wells on a 24- well culture plate coated with a monolayer of Vero cell cultures and the infected cells incubated for one hour at 37^{o} C. After incubation, one ml maintenance medium was added to culture cells, incubated at 37^{o} C and observed daily for cytopathic effects (CPE). The same samples were also inoculated in 24 well culture plates coated with a monolayer of C6/36 cell cultures and incubated at 28^{o} C. Cell cultures supernatant were harvested from the cell monolayer showing cytopathic change by scrapping off the monolayer and aspirating everything into a 1.5 ml sterile

Cryovials. The supernatant was stored at -80^{o} C for passaging and virus identification by RT-PCR. (Petz, 2014).

3.3.3 Viral RNA Extraction and cDNA Synthesis

3.3.3.1 Viral RNA Extraction

For Viral RNA extraction, 250 µL of supernatant from each of the 50 pools that were positive by cell culture was added individual 1.5 ml micro-centrifuge tube containing 750 µL Trizol-LS in an enhanced Bio Safety Level 2 cabinet (BSL2). The sample was then transferred to the RNA extraction cabinet in the extraction room; equal volumes of absolute (95%) ethanol added to each sample solution and vortexed to mix. The sample solution was then transferred into zymo-spin ^{TM} IIC column in collection tubes and centrifuged at 14000 rpm for 30 seconds. The flow-throw was discarded and the sample treated for DNase by adding 5 µL DNase I and 75 µL DNA digest buffer to each sample, mixed and incubated for 15 minutes. The column containing the sample was washed twice with 400 µL Direct-zol^{TM} and centrifuging at 14000 rpm for 30 seconds. 700 µL RNA wash buffer was then added to the column and centrifuged at 14000rpm for 2 minutes and the column transferred into 1.5ml RNA free micro-centrifuge tube. The viral RNA was eluted with 30 µL DNase/RNase-free water and frozen at -80^{o}C prior to complementary DNA (cDNA) synthesis using inhouse optimized SOP and published work (Chomczynski & Sacchi, 2006; ZYMO RESEARCH CORP., 2016)

3.3.3.2 Viral cDNA Synthesis

Viral cDNA was synthesis following an optimized inhouse SOP where; 10 µL viral RNA sample was added in a PCR tube containing 1 µL dNTP and 2 µL of random hexamer (100nmol) and mixed well. The sample was denatured in a thermos- cycler for 10 minutes at 70^{o}C followed by cooling at 4^{o}C for 5 minutes. This was followed by the addition of 2 µL DTT, 1 µL superscript reverse transcriptase, 0.25 µL RNase out and 4 µL 5X fusion buffer to the sample in the PCR tube and then incubating in a thermo cycler programmed at the following conditions; further incubation at 25^{o}C for

15 minutes, 42ºC for 50 minutes, 70ºC for 15 minutes and held at 4ºC prior to PCR amplification as described in the next section (Wolff et al., 1992).

3.3.3.3 Viral Amplification by RT-PCR

PCR amplification of the targeted arbovirus families (Table 3.1) was carried out in 25 µL reaction containing 12.5 µL of Amplitaq PCR master mix, 0.25 µL of each of forward and reverse primers (Table 3.2), 2 µL viral cDNA sample and 9.5 µL PCR water. All PCR amplifications were carried out in 35 cycles.

Table 3.1: Families PCR Amplification Conditions

Flavivirus	**Alphaviruses**
Initial heating 95ºC-10 minutes	Initial heating 95ºC-10 minutes
Denaturation 95ºC- 30 seconds	Denaturation 95ºC- 30 seconds
Annealing 55ºC- 30 seconds	Annealing 49ºC- 25 seconds
Extension 72ºC- 30 seconds	Extension 72ºC- 30 seconds
Final extension 72ºC- 7minutes	Final extension 72ºC- 7minutes
Final holding 4ºC infinity	Final holding 4ºC infinity

*The above table shows the optimized PCR conditions for flaviviruses and alphaviruses.

Table 3.2: Sequences of the Primers used, the Target gene/ Protein and Sequence Position

Virus type	Target gene/protein	Primer sequence (5'-3')	Position	Reference
Alphavirus	NSP4	VIR2052F; (5' TGGCGCTATGATGAAATCTGGAATGTT-3')	6971-6997	(Eshoo et al., 2007; Ochieng et al., 2013)
		VIR 2052R;(5' TACGATGTTGTCGTCGCCGATGAA-3')	7086-7109	
Flavivirus	NSS	FU1;(5'-TACAACATGATGGGAAAGAGAGAGAA-3')	9007-9032	(Ochieng et al., 2013)
		CFD2; (5'-GTGTCCCAGCCGGCGGTGTCATCAGC-3')	9308-9283	

*The above table contains the primer sequences used to amplify Alphavirus NSP4 and flavivirus NSS gene

3.3.4 Molecular Blood Meal Analysis

3.3.4.1 Sample Preparation and DNA Extraction

The 12 blood fed mosquitoes were dissected using sterile glass slide and sharp forceps to separate the engorged abdomen from the other parts of mosquitoes. The engorged mosquito abdomens were then placed in individually labeled Eppendorf tubes. 220 µL of 1 X PBS was added to individual Eppendorf tube with the engorged abdomen followed by 20 µL proteinase K. The abdomen was then homogenized by crushing with a sterile pipette tip to release the blood to the solution in an enhanced BSL-2. 200 µL lysis buffer was then added to individual tubes, mixed by vortexing and incubated at 56^0C for 10 minutes. 200 µL of absolute ethanol was then added and vortexed before transferring the mixture into DNase free min blood and tissue spin column (QIAGEN, Valencia, CA). The samples were centrifuged at 8000 rpm for 1 minute and the flow-through and the collection tubes discarded. The spin columns were placed in new collection tubes, 500 µL wash buffer 1 (AW1) added and centrifuged at 8000 rpm for 1 minute. The flow- through and the collection tubes were then discarded and the spin columns placed in new 2 ml collection tubes. 500 µL wash buffer 2 (AW2) was added to individual spin column and centrifuged at 14000 rpm for 3 minutes and the collection tubes with the flow-through discarded. The spin column was placed in new 1.5 ml Eppendorf tubes, DNA eluted by adding 30 µL buffer AE at the center of the spin column membrane. The sample was incubated at room temperature for one minute followed by centrifugation at 8000 rpm for 1 minute. The spin columns were then discarded and the Eppendorf containing the DNA stored at 4^0C for later use (Martínez-De La Puente et al., 2013).

3.3.4.2 Blood Source Identification by PCR

Molecular blood meal analysis was done following optimized inhouse SOPs in reference to published work by Valinsky et al 2014. Mitochondrial 12S ribosomal gene, a 500 base pair fragments were amplifying using 12S3F (5'-GGGATTAGATACCCCACTATGC-3') and 12S5R (5'-TGCTTACCATGTTACGACTT-3') primer sequence (Valinsky, 2014). The total volume was calculated for 14 samples, 1 negative and one for positive control and 1

extra for pipetting errors. 23 µL of the master mix was put in each of the labeled PCR tubes followed by addition of 2 µL of individual sample to the corresponding PCR labeled tube. The mixture was covered using PCR cover strip and amplified in a 45 cycle runs in PCR machine programmed at 980C for 1 minute, 980C for 10 seconds, 610C for 20 seconds, 720C for 30 seconds, final extension at 720C for seven minutes followed by indefinite hold at 40C (Valinsky, 2014).

3.3.5 Gel Electrophoresis and Gel Purification

During PCR amplification, a positive cDNA sample and a master mix for negative controls were included to validate results. The amplified DNA products were resolved alongside a 100-lane DNA ladder on a 2% agarose gel prepared using 1X TBE buffer and stained with 60 µL of 0.02% ethidium bromide. Electrophoresis was carried out, and DNA bands were visualized under a UV trans-illuminator (Niare et al., 2016).

Similarly, blood meal PCR products were separated on a 2% agarose gel prepared by dissolving 3 g of agarose in 150 mL of 1X TBE buffer and staining with 60 µL of 0.02% ethidium bromide. A 1200-lane DNA ladder was loaded into the first well for reference. The gel was run at 120 V for 1 hour, and the resulting DNA bands were visualized under UV light (Valinsky, 2014). Distinct bands containing target DNA were carefully excised from the gel and weighed.

Each gel slice was mixed with an equal volume (1:1 ratio) of binding buffer, incubated at 37–55°C for 10 minutes, and intermittently inverted to ensure complete dissolution. The melted gel mixture was then transferred to a Zymo-Spin™ column fitted into a collection tube and centrifuged at 14,000 rpm for 1 minute. The flow-through was discarded. The column was reinserted into the same collection tube, and 200 µL of DNA wash buffer was added. Following centrifugation at 14,000 rpm for 30 seconds, the wash step was repeated (ZYMO RESEARCH CORP., 2016).

Next, the Zymo-Spin™ column was transferred to a clean 1.5 mL DNase/RNase-free microcentrifuge tube. A volume of 10 µL DNA elution buffer was carefully applied to the centre of the column membrane, and the tube was centrifuged at 14,000 rpm

for 1 minute to elute the purified DNA. The purification column was then discarded, and the eluted DNA was stored at –80°C pending sequencing (ZYMO RESEARCH CORP., 2016).

3.3.6 Sequencing of Blood Meal DNA Samples

Sanger sequencing was performed for twelve blood-meal DNA samples together with a positive control, and the reactions were outsourced from a commercial sequencing provider. The resulting chromatograms were quality-checked, and consensus sequences were obtained. To determine host identity, the obtained sequences were queried using the Basic Local Alignment Search Tool (BLAST) against the GenBank database (Alkan et al., 2022; Benson et al., 2014; Boratyn et al., 2013).

3.4 Data Analysis

All data were entered and organized using Microsoft Excel, and statistical analyses were performed using STATA version 12 for Windows (StataCorp, 2011). The overall adult mosquito survivorship from egg collections was estimated using the ratio of the number of adults that emerged (A) to the total number of first instar larvae hatched (L_1), as described by (Lanzaro & Touré, 2004).

To assess the distribution of mosquito populations across the study sites, abundance was calculated as the ratio of mosquito species collected to the number of trapping units used (for adult traps) or the number of positive breeding habitats (for larvae). A one-way analysis of variance (ANOVA) was used to examine differences in larval productivity of *Aedes* and *Culex* species across different habitat types.

Larval density was estimated by dividing the total number of larvae collected from each habitat by the number of dips taken using a standard dipper. For habitats sampled using a pipette, one pipette collection was assumed to be equivalent to one dip for analytical purposes.

To explore potential environmental predictors of culicine larval abundance, simple linear regression analyses were conducted between larval population counts and measured environmental variables.

Mosquito species diversity and community evenness were evaluated using Shannon's diversity index (H') and Shannon's equitability index (EH). These indices were calculated using the following formulas:

$$H = -\sum_{i=1}^{s} P_i \ln(P_i)$$

$$E = \frac{e^H}{\ln(S)}$$

Where H is the Shannon's diversity index and p_i is proportion of the species relative to total number of the species. E_H is the Shannon's equitability index calculated by dividing H by the natural logarithm of total number of mosquito species within the community (richness) (Magurran, 1988; Mwangangi et al., 2009). The results were considered significant at $p<0.05$.

All statistical tests were two-tailed, and results were considered significant at $p < 0.05$. Virus Infection Rate was calculated per mosquito species per site using pooled infection Rate Program (Biggerstaff, 2006). Raw sequence chromatograms were imported and edited in Geneious Prime (Biomatters, 2025) where low-quality bases were trimmed, and consensus sequences were generated for BLAST analysis against the NCBI GenBank database.".

All sequences were subsequently compared against the NCBI GenBank database using the BLAST algorithm to determine host species identity based on sequence similarity. Only sequences with a ≥90% identity match and significant e-value (<1e–5) were considered positively identified (Alkan et al., 2022; Benson et al., 2014; Boratyn et al., 2013). The identified hosts were recorded for further ecological analysis. The phylogeny was inferred using the Maximum Likelihood (MEGA 12)

and Hasegawa-Kishino-Yano (1985) model with branch support assessed by 1000 bootstrap (Kumar et al., 2024)

3.5 Ethical Consideration

Ethical approval was obtained from the Scientific and Ethical Review Committee (SERU) of Kenya Medical Research Institute (KEMRI/SERU/CVR/04/3442) as attached on Appendix I Consent to carry out sampling within the forest ecosystem was sought from Kenya Wildlife Services and Haller Park management prior to commencement of the study.

CHAPTER FOUR

RESULTS

4.1 Larval Habitat Diversity

A total of 221 mosquito larval habitats were identified and characterized across the study sites. Sixteen different larval habitat types were identified and classified based on entomological classification standards as described in in-house standard optimized protocol (SOP). They comprised of tires, containers, roadside drainages, flower axils, house drainages, manholes, water troughs, water tanks, ditches, car track, wells, flower pot, swimming pool, puddle, climb shells and swamp. Other types of habitats sampled included scrap metals, household utensils, abandoned fountains, construction water tanks, damped polyvinyl chloride (PVC) mat, open area water collection, storm water drain, trailer, water pipe linkage, and polythene papers. Habitats encountered only once during the study period were together pooled up to make other miscellaneous aquatic habitats.

4.2 Relative Abundance and Distribution of Mosquitoes Species

4.2.1 Abundance and Distribution of Juvenile Mosquito in Urban Areas

A total of 12716 juvenile mosquitoes sampled consisting of 10472 (82.35%) larvae and 2244 (17.65%) pupae. The 10, 472 larvae were classified as early instars (48.9%) 5, 121 and late instars 5, 351(51.1%). The most productive habitat with the highest mosquito juvenile population were tires, road drainages, containers, manholes and house drainages. As shown in Figure 4.1. Linear regression showed that water turbidity and the age of the habitat were significant in prediction of Culicine larval production in an aquatic habitat (table 3). The most productive area was Mvita (44%), followed by Kisauni/Nyali (27.2%), Changamwe (22.4%), Likoni (6.5%).

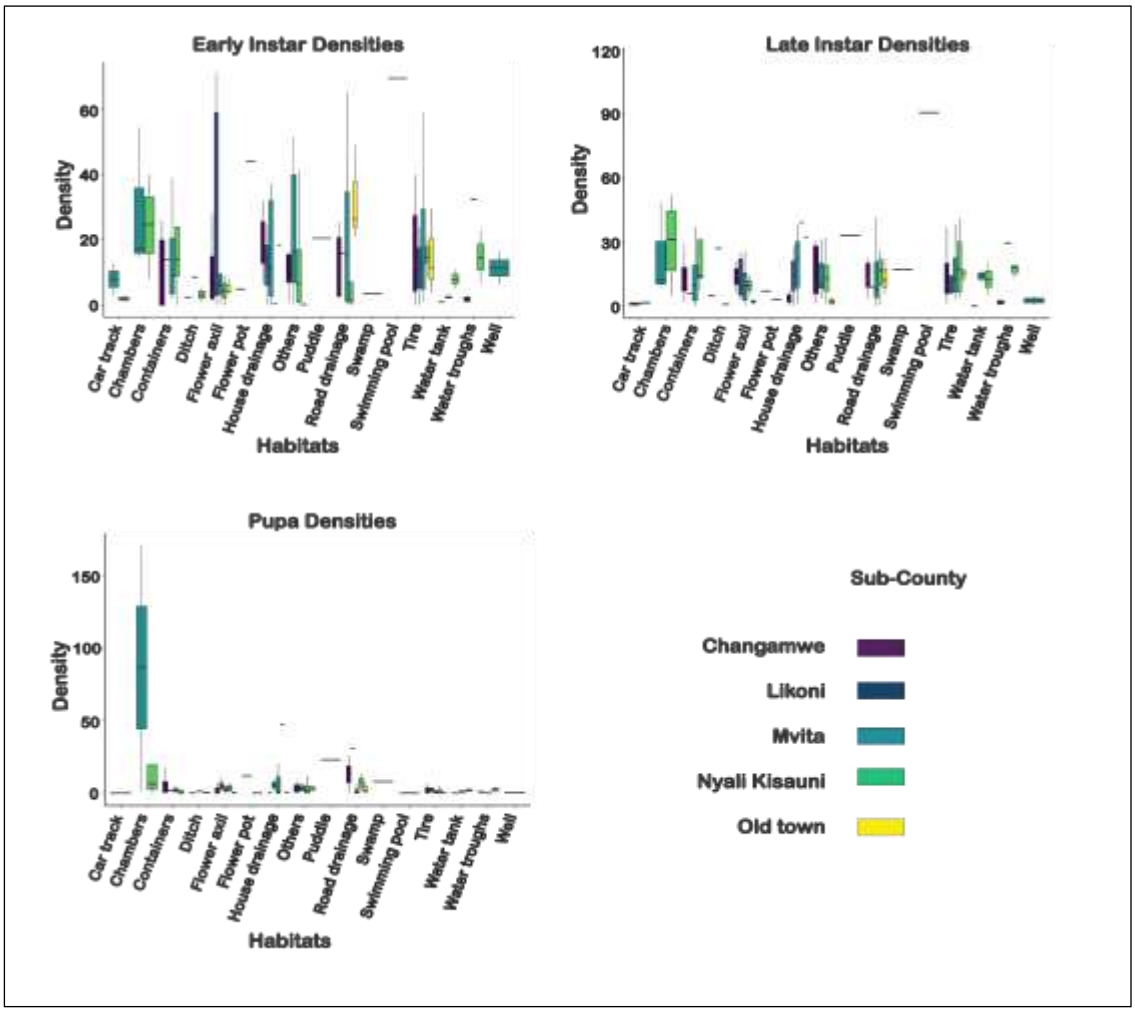

Figure 4.1: Relative Density and Distribution of Culicine Larvae and Pupae in Different Habitat Types across the Four Study Sub-Counties in Urban Areas

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Table 4.1: Linear Regression Analysis Showing Correlation between the Culicine Larval Population and the Environmental Variables

Coefficients

Model	Unstandardized Coefficients		Standardized Coefficients	T	Sig.	95.0% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
(Constant)	1.794	.630		2.848	.006	.541	3.048
Predators	.043	.067	.064	.641	.523	-.090	.176
Length	.003	.003	.115	1.175	.243	-.002	.009
Width	-.087	.056	-.171	-1.554	.124	-.198	.024
Depth	-.015	.105	-.016	-.141	.888	-.224	.194
Vegetation (%)	-.001	.005	-.025	-.255	.799	-.012	.009
Age of habitat	.176	.046	.389	3.802	**.000**	.084	.268
Land use	.064	.079	.089	.818	.416	-.092	.221
Habitat type	-.010	.009	-.123	-1.129	.262	-.027	.007
water turbidity	-.271	.073	-.382	-3.728	**.000**	-.415	-.126
Water flow	-.301	.210	-.143	-1.435	.155	-.719	.116

*B-Unstandardized Coefficient, std error- standard error of B, T; t- statistic; Sig- significance/t-value

4.2.1.1 Species Composition and Distribution among the Different Aquatic Habitats in Mombasa Island and its Environs

Aedes aegypti and *Culex quinquefasciatus* were the most dominant species found across Mombasa Island and its environs during the study period (Figure 4.2). The 12,716 Culicine larvae sampled were taxonomically identified to eight species namely; *Cx. quinquefasciatus* (50.17%), *Ae. aegypti* (38.73%), *Ae. vitattus (5.2%), Ae. simpsoni (3.77%) and Ae. argenteopantatus, Cx. annulioris, Cx. tigripes,* and *Tx. brevipalpis* recording less than 1% each. Figure 4.3 shows Culicine species distribution in various aquatic habitats in the four sub-counties. The five commonly distributed species across the four sub-counties during this study period were *Cx. quinquefasciatus, Ae. aegypti, Ae. vittatus, Ae. simpsoni* and *Cx. tigripes*. The rest three species were only found in Changamwe sub-county. In Mvita, the most common species were *Cx. quinquefasciatus* (58.9%) and *Ae. aegypti* (37.1%), in Changamwe *Ae. aegypti* (54.2%) and *Cx. quinquefasciatus* (31.4%), Nyali *Cx. quinquefasciatus* (49.8%), *Ae. aegypti* (34.6%) and *Ae. vittatus* (13.7%), Likoni *Cx. quinquefasciatus* (57.0%), *Ae. simpsoni* (27.9%) and *Ae. aegypti* (16.1%) the rest recorded less than 5% in each of the study site.

Ae. aegypti was the dominant population in containers, water tanks, tires and flower pots. Only *Aedes aegypti* species were sampled along the beach line of the Indian Ocean during the study period. Nyali/Kisauni recorded higher proportions of *Cx. quinquefasciatus* (27.00%) contrast to Changamwe (14.00%). They dominated in ditches, house drainages, manholes, road drainages and water troughs and none of *Cx. quinquefasciatus* was sampled from swamp, flower axils and swimming pools.

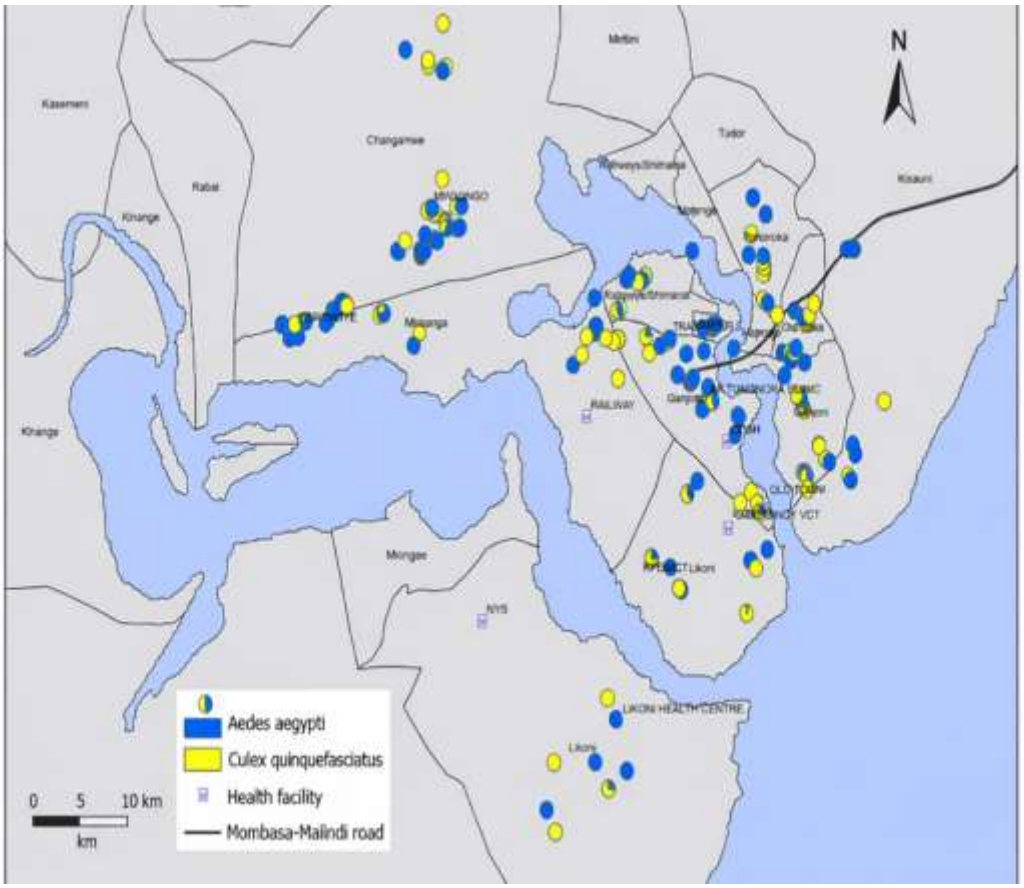

Figure 4.2: *Culex Quinquefasciatus or Cx. Pipiens and Aedes Aegypti* Density and Distribution in Mombasa and its Environs

*The two species are relatively distributed across the urban centers

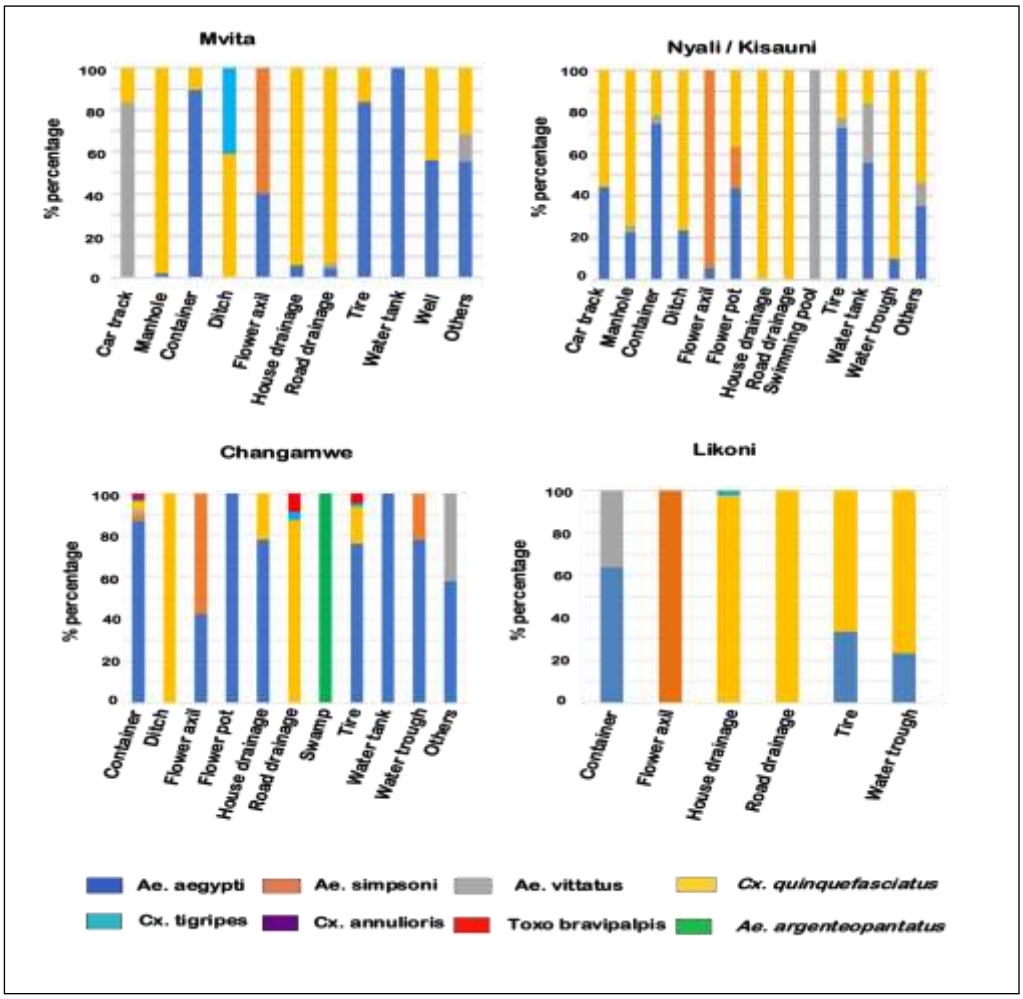

Figure 4.3: Culicine Mosquito Species Distribution within Mombasa Island and its Adjacent Mainland

**Cx. quinquefasciatus/Cx. pipiens* were the most predominant in Mvita, Nyali and Likoni. *Ae. aegypti* were dominant in Nyali

4.2.2 Relative Abundance and Distribution of Mosquito Species in Haller Park, Arabuko, Gede and Bamburi

A total of the 4675 adult and 353 juvenile mosquitoes belonging to 19 species were sampled in Haller Park, Arabuko Sokoke forest, Gede and Bamburi between November and December 2016. Haller Park recorded the highest mosquito population (87.2%) followed by Bamburi (7.9%), Gede (4.0%) and Arabuko Sokoke forest (0.9%). The highest diversity was recorded in *Aedes* genus with 8 species,

followed by *Culex* (5), *Anopheles* and *Mansonia* had 2 each, *Eretmapodite* and *Ficabia* (1 each). *Ae.tricholabis (45.2%), Ae. aegypti (20.0%), An. funestus (13.9%) Cx. pipiens (6.5%) Cx. vansomereni* (4.5%) *Cx. univittatus* (2.8%) and *Ae. vittatus* (1.9%) were the most predominant species sampled across the four study sites during the study period as shown in Figure 4.4. Other species including *Ae. chausseri, Ae. simpsoni, Er. chrysogaster, Ma. africanus, Ma. uniformis, An. coustani, Ae. mcintoshi, Ae. hirsutus, Ae. tarsalis, Cx. annulioris, Cx. tigripes, Ficalbia mediolineata* and unidentified species recorded less than 1% each.

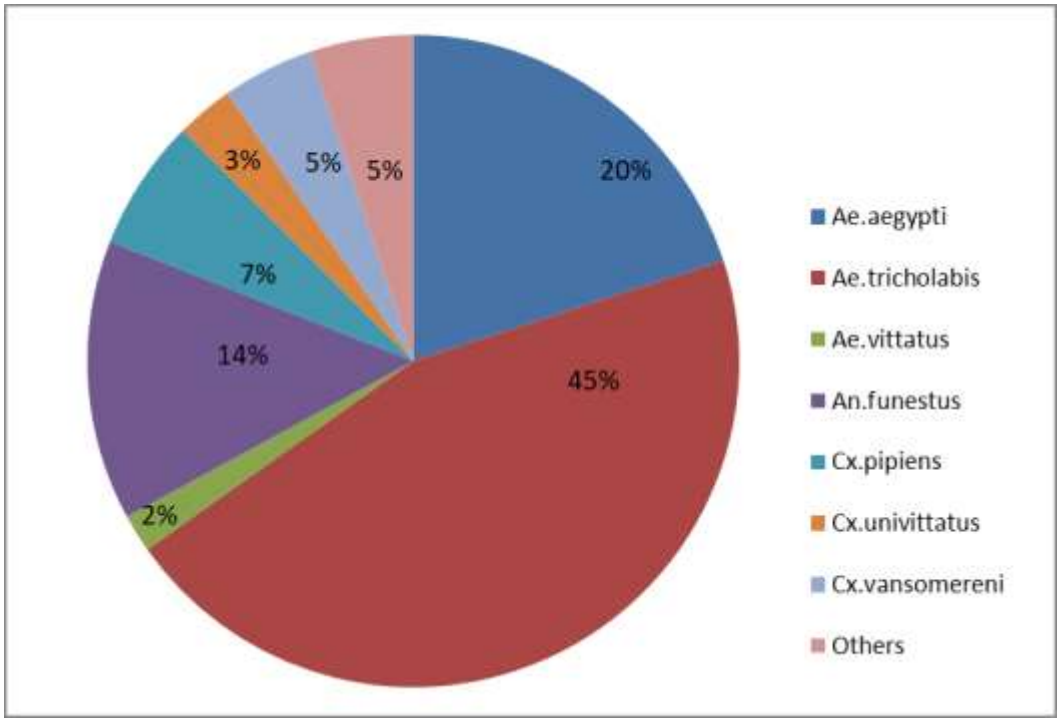

Figure 4.4: Relative Abundance of Mosquito Species across the Forested and Peri-urban Sites

**Ae. trichilabis* and *Ae. aegypti* which are both vector of arboviruses were the most predominant across the study sites

Ae. aegypti and *Ae tricholabis* were the only species distributed across the four study sites durigh the study period. Haller Park recoded the highest species diversity (18) with Arabuko Sokoke recording the list (3). Bamburi and Gede had 10 and 7 species respectively. In Haller Park, *Ae. tricholabis* (51.4%), *Ae. aegypti* (15.7%), *An. funestus* (12.0%), *Cx. pipiens* (5.6%), *Cx. vansomereni* (4.9%), *Cx. univittatus* (3.0%), *Ae. vittatus* 1.7%), *Cx. annulioris* and *Ae. simpsoni* (1.1%) each and *Ficalbia*

mediolineata (1.0%) were the most predominant species with the other 8 species recording less than 1% each. *An. funestus* (43.9%), *Ae. aegypti* (32.3%), *Cx. pipiens* (8.6%), *Ae. vittatus* (5.6%), *Ae. tricholabis* (4.0%), *Cx. vansomereni* (2.5%) and *Cx. univittatus* (2.0%) were the most predominant in Bamburi, *Ae. aegypti* (70.6%), *Cx. pipiens* (24.4%), *Ae. tricholabis* and *Cx. univittatus* (1.5%) each in Gede and *Ae. aegypti* (85.1%), *Ae. chauseri* (12.8%) and *Ae. tricholabis* (2.1%) in Arabuko Sokoke forest. The rest of species recorded less than 1% in each study site during the study period as shown in Figure 4.5.

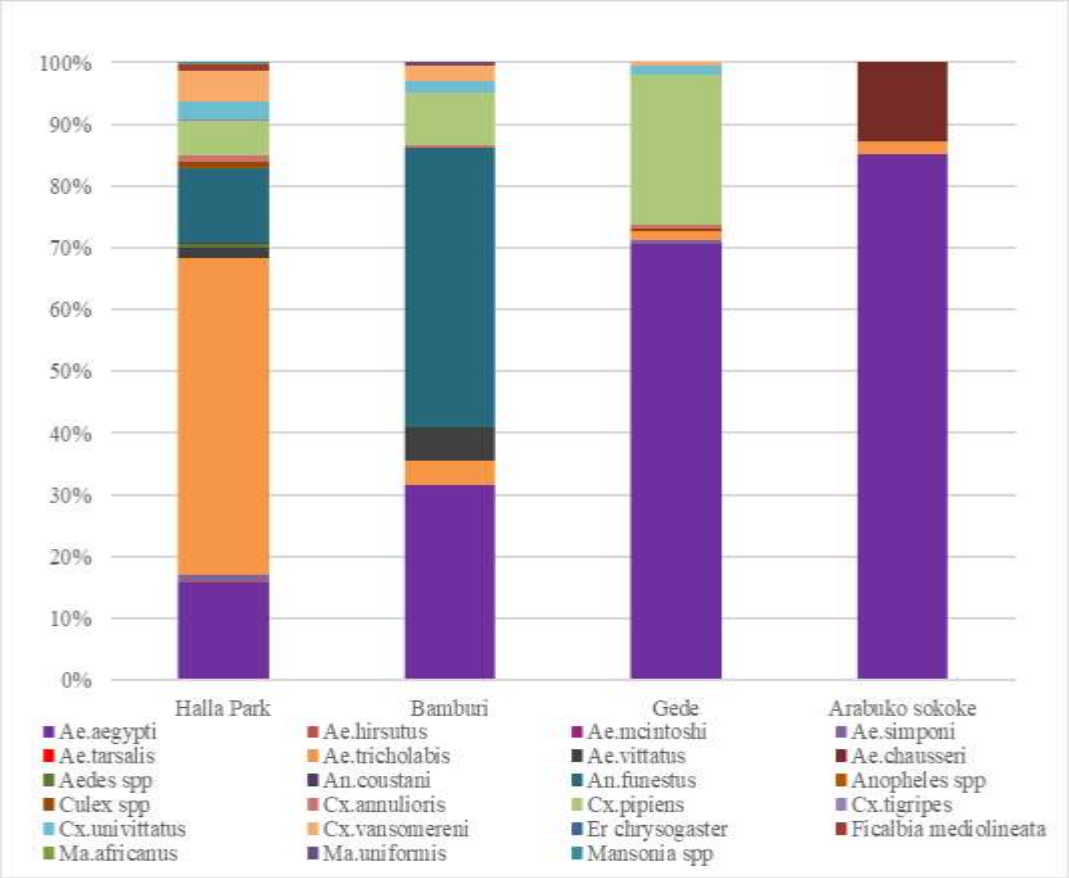

Figure 4.5: Relative Abundance and Distribution of Mosquito Species per Site

**Ae. tricholabis* and *Ae. Aegypti* were the only two species distributed across the four study sites. *Ae. chausseri* was only found in Arabuko Sokoke forest.

4.2.3 Species Diversity and Evenness Mombasa Island and its Environs

Shannon diversity index showed that mosquito species diversity (H) and evenness (E_H) was highly significant in Changamwe (H=1.208, E_H=0.581) compared to the other three sub-counties (H=1.068, E_H= 0.664 Nyali/Kisauni; H=1.014, E_H= 0.630,

Likoni and H=0.849, E_H= 0.527, Mvita). This suggests that there was a larger number of species present in Changamwe sub-county and the individual species were distributed evenly among other species in the community (Table 4.2).

Table 4.2: Culicine Species Diversity and Evenness in Mombasa Island and its Adjacent Mainland

	Sub-counties	***Aedes* species**	**Non-*Aedes* mosquito species**	**All mosquito species**
Shannon's Diversity Index (H)	Changamwe	0.548(4) *	0.549(4)	1.208(8)
	Likoni	0.728(3)	0.038(2)	1.014(5)
	Mvita	0.325(3)	0.073(2)	0.849(5)
	Nyali/kisauni	0.740(3)	0.023(2)	1.068(5)
Shannon's Equitability (E_H)	Changamwe	0.396(4)	0.396(4)	0.581(8)
	Likoni	0.663(3)	0.055(2)	0.630(5)
	Mvita	0.296(3)	0.105(2)	0.527(5)
	Nyali/kisauni	0.674(3)	0.033(2)	0.664(5)

*The values in parenthesis represent the number of species collected in the study

4.2.4 Species Diversity and Evenness in Forested and Per-urban Areas

Shannon diversity index showed that mosquito species diversity (H) and evenness (E_H) was highly significant in Haller Park (H=1.571, E_H=0.544) and Bamburi (H=1.456, E_H= 0.632) compared to the other two sites; (H=0.793, E_H= 0.408, Gede and H=0.482, E_H= 0.439, Arabuko Sokoke). This suggests that there was a larger number of species present in Haller Park and the individual species were distributed evenly among other species in the community (Table 4.3).

Table 4.3: Culicine Species Diversity and Evenness in Haller Park, Gede, Bamburi and Arabuko Sokoke Forest

	Site	*Aedes* species	Non-*Aedes* mosquito species	All mosquito species
Shannon's Diversity Index (H)	Haller Park	0.729(7) *	1.581(11)	1.571(18)
	Gede	0.141(3)	0.397(4)	0.793(7)
	Bamburi	0.684(3)	0.835(7)	1.456(10)
	Arabuko	0.482(3)	0.00(0)	0.482(3)
Shannon's Equitability (E_H)	Haller Park	0.374(7)	0.659(11)	0.544(18)
	Gede	0.128(3)	0.286(4)	0.408(7)
	Bamburi	0.632(3)	0.429(7)	0.632(10)
	Arabuko	0.439(3)	0.000(0)	0.439(3)

*The values in parenthesis represent the number of species collected in the study

4.3 Potential Vectors of Arboviruses

4.3.1 Viral Isolates by Cell Culture

Out of 637 pools inoculated C6/36 (*Aedes albopictus*) cell lines and Vero cell line 200 pools were passaged in C6/36 and 50 positive passage 3 harvested and stored at -80^{o} C for further processing. Figure 4.6 shows a negative C6/36 cell line (A) and a positive C6/36 cell line (B) as observed under the microscope in the laboratory**.**

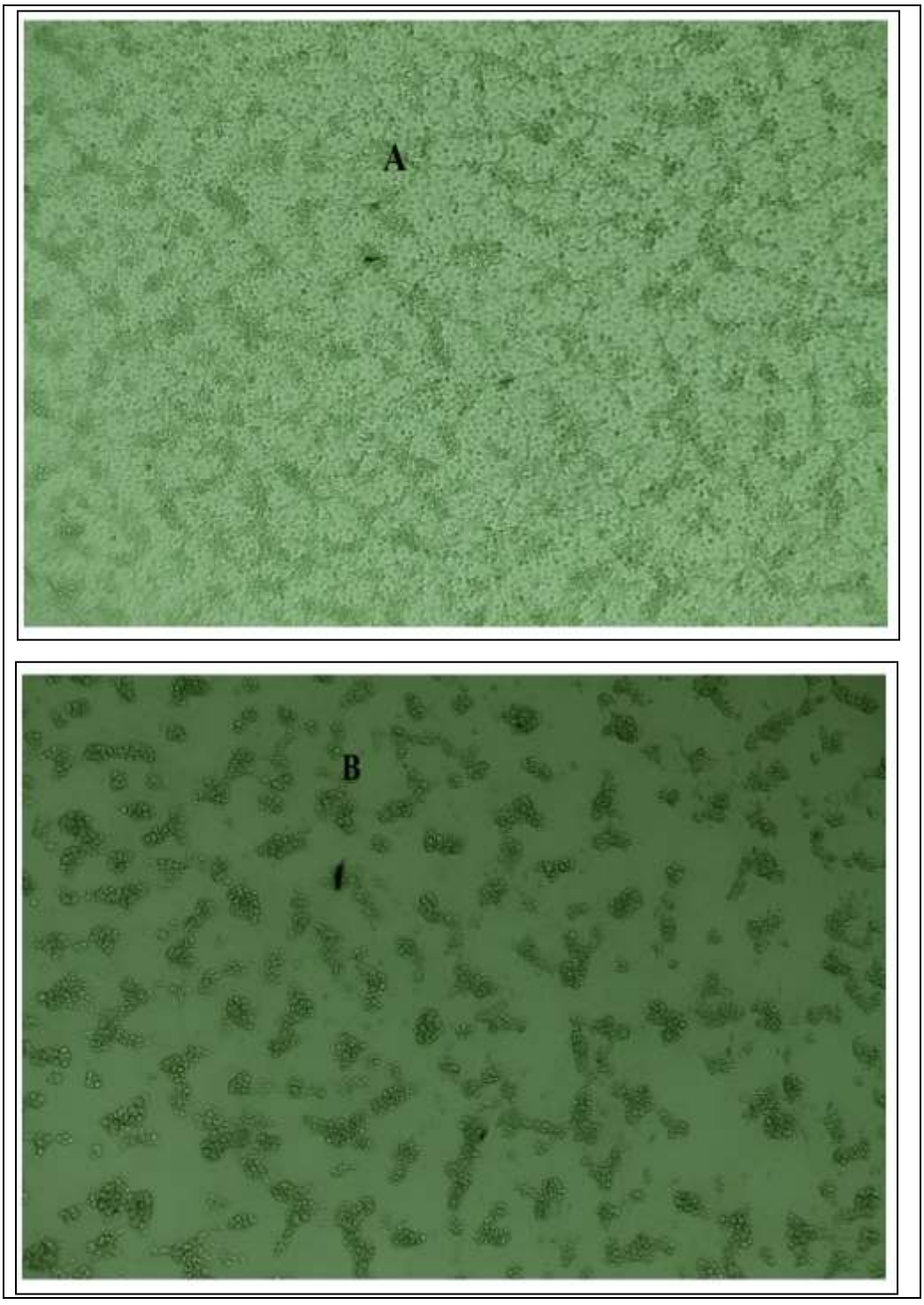

Figure 4.6: Images of C6/36 Cell Line as Viewed Under the Microscope

*A shows a negative cell line that has not been infected and B shows a positive cell line that has been infected and cells have piled off the plate as observed under microscope under X40

4.3.2 Viral Isolates by Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Out of 50 viral cDNA tested for bunya virus, flaviviruses and Alphaviruses, 23 were positive by PCR for flavivirus and 6 for alphavirus. Haller Park reported the highest numbers with 16(55.2%) flaviviruses and 6(20.7%) alphaviruses followed by Bamburi with 5(17.2%) flaviviruses and Gede with 2 (6.9%) flaviviruses. No positive samples were reported from Arabuko Sokoke and Mombasa and its environs during this study as shown in table 6 below. *Ae. aegypti* reported the highest number of infections across the three sites with 6 (27.27%) in Haller Park, 3(60%) in Bamburi and 2 in Gede (100%). The two pools from Gede were from larval collection**.** *Ae. vittatus* and *Ae. tricholabis* recorded significant infection rate with 4 (13.8%) positives each. *Ae. vittatus* 2(40%) flaviviruses in Bamburi, 1(4.5%) flavivirus and 1(4.5%) alphavirus in Haller Park while *Ae. tricholabis* recorded 3(13%) flaviviruses and 1(4.5%) alphavirus in Haller Park. Figure 4.7. Contain agarose gel electrophoresis images of the amplified arboviruses.

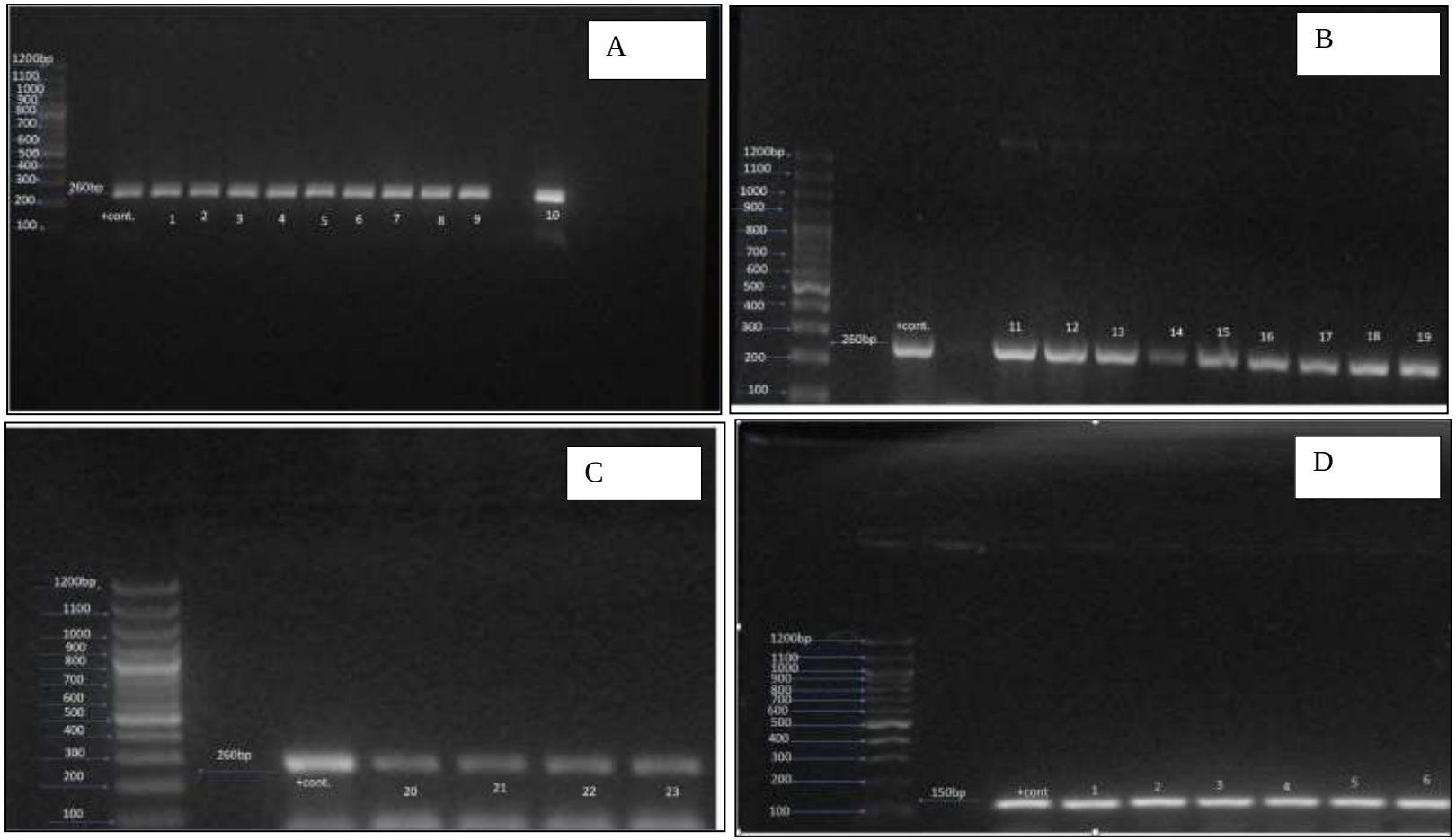

Figure 4.7: Agarose Gel Electrophoresis Showing Flaviviruses and Alphaviruses Amplification from Mosquito Samples

*A, B & C are gel images of flaviviruses. D is a gel image of alphaviruses. A: Lane L1: 1200 bp ladder; L2 negative control, L3 positive control; L4-L14 clear bands of 260 bp for flaviviruses and D L1 1200bp ladder, L2 negative control, L3 positive control and L4-L9 positive bands of 150bp for alphaviruses indicate successful amplification of the arbovirus

Table 4.4: Arbovirus Infection Rate Distribution across the Study Sites

Sites	Species	Count of Flavivirus	Count of Alphavirus	Infection rate
Bamburi		5(21.74%)		
	Ae. aegypti	3 (60%)		0.05
	Ae. vittatus	2(40%)		0.03
Gede		2(8.70%)		
	Ae. aegypti	2 (100%)		0.04
Haller Park		16(69.56%)	6(20.69%)	
	Ae. Hirsutus	1(4.5%)	0	0.002
	Ae. aegypti	5(22%)	1(4.5%)	0.012
	Ae. tricholabis	3(13%)	1(4.5%)	0.008
	Ae. vittatus	1(4.5%)	1(4.5%)	0.004
	An. funestus	3(13%)	0	0.006
	Cx. annulioris		1(4.5%)	0.002
	Cx. pipiens	2(9.1%)	0	0.004
	Cx. vansomereni		2(9.1%)	0.004
	Fi. Circumtesta	1(4.5%)	0	0.002
Grand Total		23(79.31%)	6(20.69%)	

*The values in parenthesis represent percentages of infected pools per site

4.4 Links between Sylvatic, Intermediate and Urban Transmission Cycles

4.4.1 Relative Abundance and Species Distribution across the Three Eco Zones

Relative abundance profiles showed clear differences in mosquito community composition across settlement types (Figure 4.8). Urban sites were dominated by a few taxa, most notably *Culex pipiens* and *Aedes aegypti*, which accounted for most specimens collected, with only minor contributions from other species. Peri-urban sites contained a more mixed assemblage, with *Ae. aegypti* still common but co-occurring with *Anopheles funestus* and several other species at moderate frequencies. In contrast, forest sites supported a broad range of species, with *Aedes tricholabis* overwhelmingly dominant and several species contributing moderately to the overall community. Presence of common arbovirus vectors such as *Ae. aegypti*, *Ae. simpsoni*, *Ae. vittatus* and *Cx. pipiens* across the three eco-zones is a clear indication of a potential link between the sylvatic and urban cycles with an active intermediate cycle (Table 4.4 above. Further, presence of *Ae. aegypti* and *Ae. tricholabis* in both

forested and peri-urban, and their ability to feed on both animals and humans suggest there is a clear link between the sylvatic and urban transmission through spillover.

These patterns were reflected in the diversity indices as shown in table 4.5 below, Forest sites supported the broadest range of species (20 taxa) and displayed the highest diversity (Shannon index = 2.37; Simpson index = 0.69), indicating both richness and balanced species composition. Peri-urban sites harbored an intermediate assemblage (12 taxa; Shannon index = 2.08; Simpson index = 0.69), while urban sites were characterized by lower richness (8 taxa) and diversity (Shannon index = 1.59; Simpson index = 0.59), reflecting dominance by a few species. Evenness values (0.53–0.58) suggest that although all settlements exhibited some dominance by common taxa, forest and peri-urban sites maintained more balanced species distributions compared with urban habitats. These results demonstrate a clear gradient of declining mosquito diversity from forest to urban settlements.

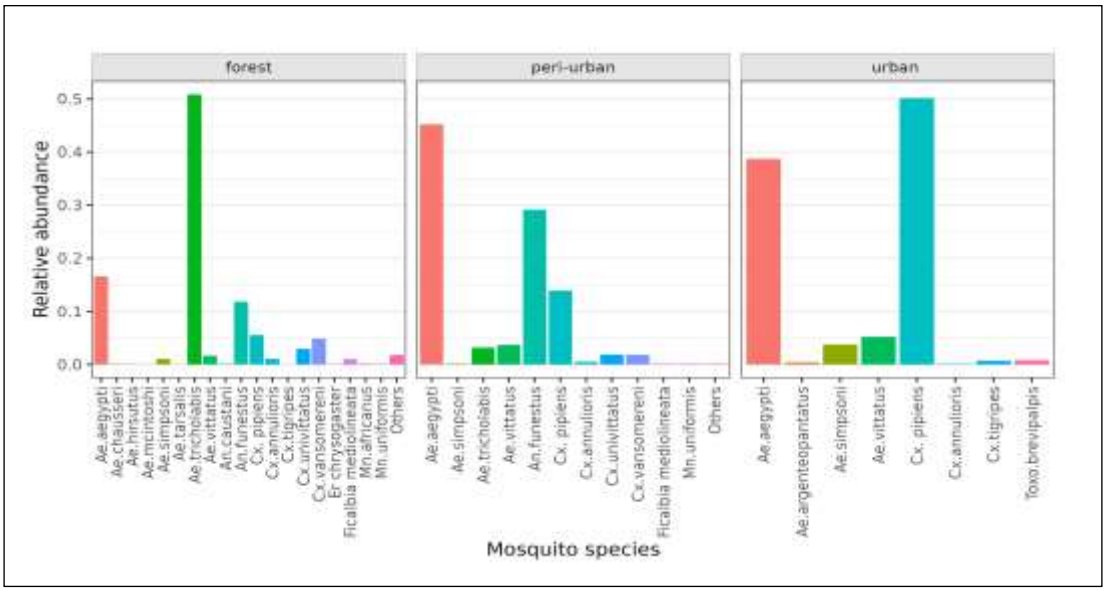

Figure 4.8: Relative Abundance and species distribution across the Three eco zones

Ae. tricholabis were the most predominant inforest, *Ae. aegypi* were predominant in peri-urban while *Cx. pipiens* were the most predominant in urban. Aedes aegypti were distributed across the three eco-zones

Table 4.5: Mosquito Species Diversity and Evenness across the Three Eco-zones

Settlement	Shannon Index	Simpson Index	Pielou's Evenness	Species Richness
Forest	2.37	0.69	0.55	20
Peri-urban	2.08	0.69	0.58	12
Urban	1.59	0.59	0.53	8

*Forest recorded the highest species diversity with Shannon index of 2.37

4.5 Host-Feeding Patterns, Phylogenetic Validation, and Vector–Host Connectivity

4.5.1 Mitochondrial Ribosomal Gene Sequence

All the 12 blood fed samples were from Light traps collected at Haller Park. The 12 samples aligned against *Otolemur crassicaudatus* (brown great galago) from previous studies and 1200 bp ladder (Figure 4.9). The first well is a negative control followed by positive control and samples in the order 1-12. Out of the 12 samples 8 were identified positively, 4 remained unassigned. The animals identified include *Ichneumia albicauda* (white tailed mongoose)-1, Tragelaphus scriptus (bushbuck)-2, Giraffa reticulata (Somali giraffe)-4, *Oryx beisa (1)* and Sylvilagus floridanus (eastern cottontail)-1. Out of the 12 blood fed samples 10 were *Ae.tricholabis*, 1 *Ae. aegypti* and 1 *Cx. watti* as shown in table 8 below. Individual sample Pairwise sequence alignment can be referred to on Appendix III and individual blood source phylogenetic.

The identified host species represented a diverse range of wild and domestic mammals, including *Ichneumia albicauda* (white-tailed mongoose), *Tragelaphus scriptus* (bushbuck), *Giraffa reticulata* (reticulated giraffe), *Oryx beisa* (Beisa oryx), and *Sylvilagus floridanus* (eastern cottontail). *Aedes tricholabis* exhibited the broadest host range, feeding on multiple wild mammal species, indicating its opportunistic feeding behaviour and potential role as a bridge vector between wildlife and human environments. In contrast, *Aedes aegypti* and *Culex watti* were each associated with a single host species. Overall, the network highlights a complex

pattern of mosquito–host interactions, dominated by *Ae. tricholabis* with multiple connections to wild vertebrates, suggesting ecological flexibility and a possible role in zoonotic arbovirus maintenance within sylvatic cycles as shown in a bipartite mosquito- mammals interaction network in Figure 11.

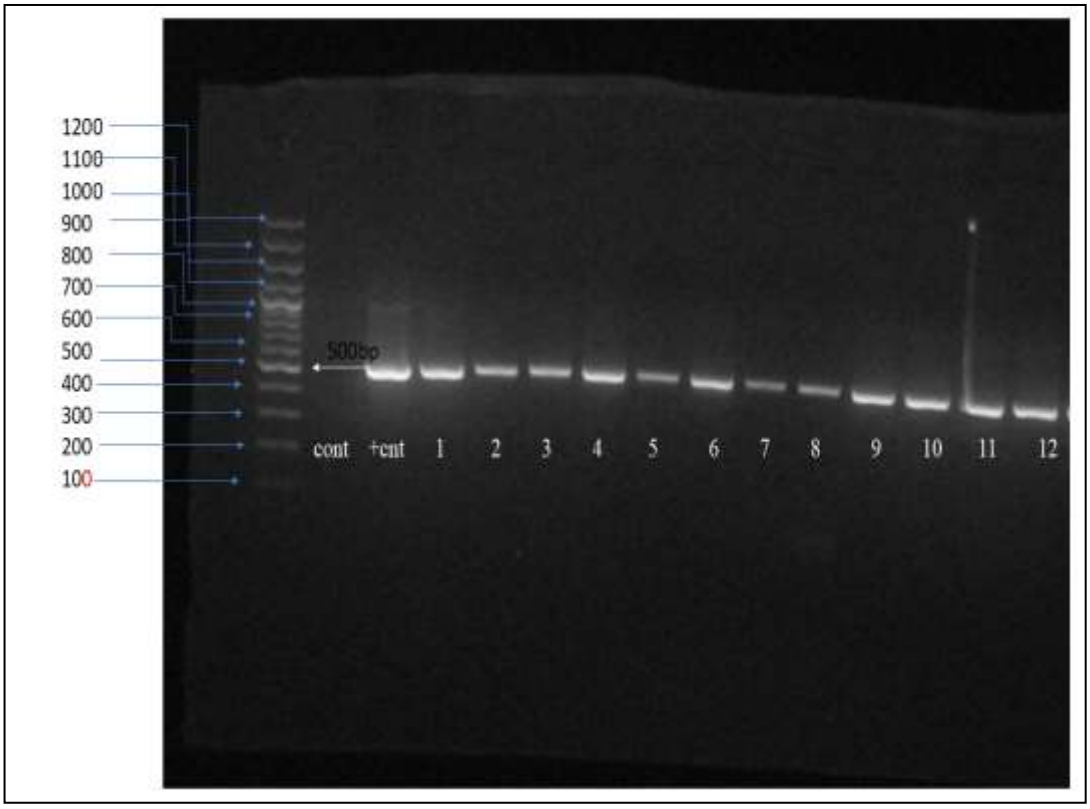

Figure 4.9: Gell Image Showing Blood Mean PCR Samples against Controls

*- cont. refers to negative control which included only the PCR master mix, +cont. refers to

Table 4.6: Blood Source Identification by BLAST and BLAST Accession Number

Site of collection	*Mosquito Id*	*Scientific Name*	Local name	Accession number	E. value	Per. Ident.
1	*Ae. aegypti*	*Unknown*	_	_	_	_
2	*Ae. tricholabis*	*Ichneumia albicauda*	White-tailed mongoose	NC_053967.1	0.0	97.12%
3	*Ae. tricholabis*	*Tragelaphus scriptus*	Harnessed bushbuck	ON474901.1	4e-96	91.10%
4	*Cx. watti*	*Tragelaphus scriptus*	Harnessed bushbuck	ON474901.1	0.0	99.29%
5	*Ae. tricholabis*	*Oryx beisa*	Oryx beisa	NC_020793.1	0.0	98.58%
6	*Ae. tricholabis*	*Unknown*	_	_	_	_
7	*Ae. tricholabis*	*Giraffa tippelskirchi*	giraffe	OM973996.1	1e-45	86.73%
8	*Ae. tricholabis*	*Unknown*	_	_	_	_
9	*Ae. tricholabis*	*Unknown*	_	_	_	_
10	*Ae. tricholabis*	*Giraffa cameloparalis*	giraffe	MT605025.1	0.0	99.55%
11	*Ae. tricholabis*	*Giraffa cameloparalis*	giraffe	OM974024.1	0.0	99.55%
12	*Cx. univitattus*	*Giraffa tippelskirchi*	giraffe	OM973996.1	1e-94	99%
13	_	*Otolemur crassicaudata*	Brown great galago	NC_012762.1	0.0	98.96%

*Uknown samples are those that failed to identify by BLAST analysis due to DNA degradation, low template concentration, mixed blood meals, poor sequence quality, or the absence of representative reference sequences in public databases.

4.5.2 Evolutionary Analysis of Blood Meal Sources by the Maximum Likelihood Method

Maximum Likelihood phylogenetic analysis of mitochondrial DNA sequences supported the vertebrate host identifications obtained from sequence similarity searches based on crustalW alignment sequence on appendix III. Blood meals from the most frequently detected host, *Giraffa* spp., clustered into closely related clades with high bootstrap support (90–99%), despite moderate intraspecific variation. Blood meals from *Tragelaphus scriptus* also formed a well-supported cluster (94%). Less frequently detected hosts, including *Oryx* spp., *Otolemur* spp., and mongoose, each formed distinct lineages with strong bootstrap support (96–99%), confirming reliable host identification across all samples Figure 10 and individual blood source phylogenetic trees can be referred on from Appendix V.

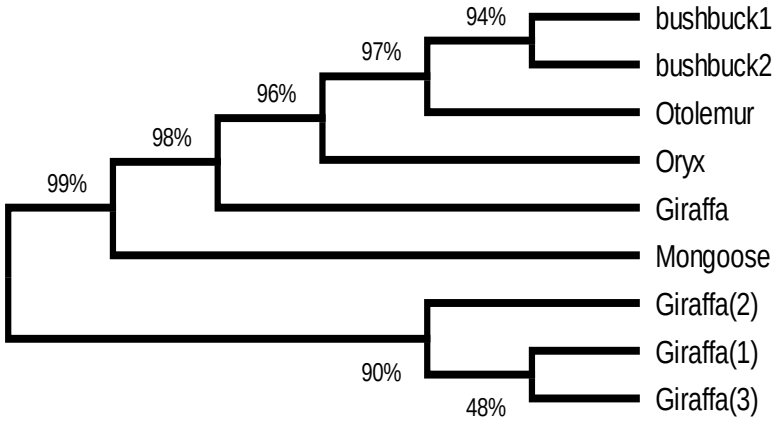

Figure 4.10: Blood Source Phylogenetic Tree Based on 12S Mitochondrial Gene

*Maximum Likelihood phylogenetic tree generated in MEGA X based on 12S mitochondrial gene sequences obtained from mosquito blood-meal samples. Bootstrap support values (1,000 replicates) are indicated at the corresponding nodes. The tree shows clustering of sequences according to their vertebrate host species, with strong support observed for major clades, including *Giraffa* spp. and *Tragelaphus scriptus* (bushbuck).

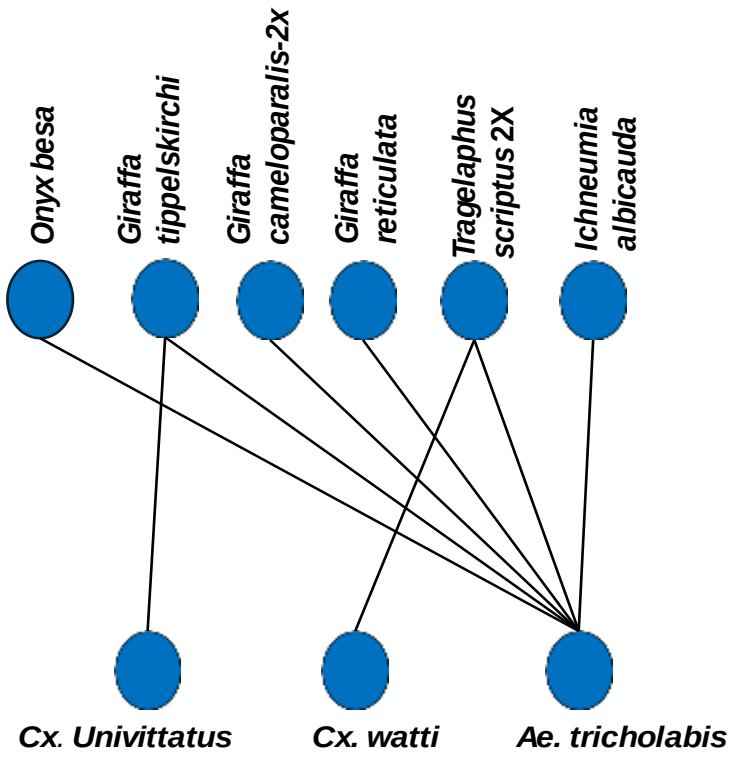

Figure 4.11: Mosquito–Host Interaction Network Depicting Blood-Feeding Connections across Mosquito Vectors and Mammalian Hosts

*The host network demonstrates the ability of the *Ae.tricholabis* to feed on diverse wild animals in the forest

CHAPTER FIVE

DISCUSSION

5.1 Relative Abundance and Distribution of Mosquito Species Associated with Arbovirus Transmission

The findings of this study demonstrate significant ecological and spatial variation in mosquito abundance and diversity across urban, peri-urban, and forested habitats in the coastal region of Kenya. These variations reflect differences in environmental characteristics, human activity, and habitat availability, all of which influence mosquito breeding and survival. A total of 221 mosquito larval habitats were identified and characterized, indicating a high diversity of aquatic habitats supporting mosquito proliferation across the study areas. Majority of mosquito species co-existed in diverse habitat types with exception to *Ae. argenteopantatus* that was found to occur singly in a swamp. This shows that different mosquito species share nutrients resources within their habitats which ensure continuous production and emergence of the adult mosquito species throughout the year (Mwangangi et al., 2008; Trpis', 1972). The current study reported diverse breeding habitats for *Ae.aegypti* compared to previous studies that described them mainly in tires and containers (Midega et al, 2006; Lutomiah et al., 2016). Other culicines mosquitoes were found to breed in a wide range of breeding habitats although in small numbers. *Ae. simpsonis* predominantly bred in flower axils similar to the observation that had be recorded previous in a mosquito larval surveillance studie in Tanzania and Uganda (Lutwama, 1998; Trpis', 1972). Smaller number of *Ae. simpsoni* was found to co-exist with other species in containers, water troughs, tires and flowerpots although in decreasing order of abundance. All breeding habitats supported all stages of larvae from first instars to pupae, indicative of their ability to attract gravid mosquitoes for oviposition and successfully supporting development of immature stages to adult mosquitoes. Given their significance and contribution to mosquito species diversity and distribution, these habitats should be primarily target for the vector control along the coastal Kenya (Noori et al., 2015).

In this study, egg collection was only done in forested areas where human interference was minimal, given urban and peri-urban are human populated areas. Majority of the eggs were half hatched and probably the embryo died or entered dormancy on transit contributing to low hatching rate in the insectary. Other factors could have been due inability to adapt to change of environmental factors, water and time it took to get to KEMRI Nairobi insectary. This is consistent with other studies which have shown that the eggs hatching rate decreases with varying oxygen concentration in water and also with time between egg deposit and dispensation in water (Zheng et al., 2015). In our case where eggs were dried and shipped in envelops, studies have shown that storing eggs in relative high humidity to prevent desiccation but lower enough to avoid hatching while on transit or storage could increase rate of viability or hatching (Imbahale et al., 2012; Noori et al., 2015; Trewin et al., 2017).

Larval sampling targeted all mosquito species but only culicines and toxorynchyte species were collected during the study period. Given the study was conducted during extended dry season and majority of breeding grounds were stagnant and drain water, this could explain the absence of anopheles mosquito larvae. *Aedes aegypti* and *Culex pipiens* larval production was found to be high in both urban and peri-urban areas. *Ae. aegypti* being the most predominant species in peri- urban with 80.2% and the second highest in urban with 38.7%. *Cx. pipiens* was the most predominant in peri-urban with 50.2% and the second highest in peri- urban with 17.1%. *Ae. vitattus* species led in forested area with 80.0% followed by *Ae. aegypti* at 10.5%. This is probably due to high number of suitable breeding habitats for the *Aedes* and *culex pipiens* mosquito larvae in these areas. Among these habitats, containers and tires were found to be important breeding habitats for *Aedes aegypti* while road side drainages, house drainages and man holes were found to play significant role in *Cx. pipiens* larvae production. This is consistent with other studies conducted in Malindi and Mombasa that found containers and tires to be important breeding habitats for *Ae. aegypti* larval production in urban areas (Midega et al, 2006; Karuitha et al., 2019; Lutomiah et al., 2016). *Ae. aegypti* larvae occurrence in oceanwater filled containers, well and tires along the beach line of Indian Ocean indicates that they can tolerate high saline levels compared to other species. This is in

agreement with a previous laboratory study that found coastal *Ae. aegypti* to be more adaptive compared to their plateau counterparts (Jude et al., 2012; Karuitha et al., 2019; Marylene de Bristo Arduino, 2015).

The dominance of artificial containers in urban habitats reflects the high degree of anthropogenic influence, which provides abundant and stable breeding grounds for container-breeding mosquitoes such as *Aedes aegypti* and *Culex pipiens*. These findings are consistent with previous studies in Mombasa and Nairobi that reported similar trends in mosquito productivity associated with man-made containers (Karuitha et al., 2019; Lutomiah et al., 2013, 2016; Ngugi et al., 2017). The regression analysis revealed that water turbidity and habitat age were significant predictors of culicine larval productivity, suggesting that older, organic-rich habitats favour mosquito development (Mwangangi et al., 2008). Immature *Culex* were mainly found to colonize potential breeding habitats polluted with sewage, sand, garbage and mud compared to *Aedes* species which were mainly found in fairly clean and unpolluted water. Similar studies found *Cx. pipiens* larvae breeding to be favored by organically polluted water, an indication that this polluted water produces chemical cues to attract gravid *Cx. pipiens* to lay eggs and the nutrient rich polluted water support successful development of the immature stages (Noori et al., 2015).

Urban areas typically experience increased human-vector interaction due to population density and poor waste management, which enhances arboviral transmission potential. The predominance of *Aedes aegypti* in these localities is particularly concerning, as it is the primary vector of dengue, chikungunya, and yellow fever viruses (Gubler, 2011). These results underscore the need for targeted vector control in urban centres, focusing on elimination of artificial containers and improved sanitation to reduce breeding sites.

Peri-urban environments exhibited high mosquito diversity with a mix of sylvatic and domestic species. In these sites, 12 mosquito species were identified, with Bamburi recording 10 species and Gede 7 species. *Anopheles funestus*, *Ae. aegypti*, *Cx. pipiens*, and *Ae. vittatus* were the predominant species in Bamburi, while *Ae. aegypti*, *Cx. pipiens*, and *Ae. tricholabis* were dominant in Gede.

The presence of both *Anopheles* and *Aedes* species in peri-urban habitats demonstrates ecological overlap between malaria and arbovirus vectors. This overlap may increase the complexity of vector control efforts and the potential for co-transmission of vector-borne diseases (Adeleke et al., 2010). The combination of domestic water storage practices, vegetation cover, and intermittent flooding in these areas creates diverse microhabitats that sustain both culicine and anopheline species (Kweka et al., 2012).

The high population of *Ae. aegypti* in urban and peri-urban, a vector for urban transmission and amplification of of majority of arboviruses including dengue fever and chikungunya poses a great risk of outbreaks along the coastal Kenya in an event of a sylvatic spill-over (Diallo D. et al., 2014; Gubler, 2011; Lutomiah et al., 2016).

These transitional environments act as ecological bridges between urban and forested systems, supporting both human-adapted and sylvatic mosquito species. The detection of *Ae. tricholabis* and *Ae. vittatus*, which are often associated with sylvatic cycles, suggests the potential for arbovirus spillover from wildlife reservoirs into human populations (Diallo D. et al., 2012; Diallo D. et al., 2012).

Forested areas, including Haller Park and Arabuko Sokoke Forest, displayed distinctive mosquito assemblages dominated by *Aedes* species. Haller Park recorded the highest mosquito population overall (87.2%) and the greatest species diversity (18 species), whereas Arabuko Sokoke Forest had the lowest abundance (0.9%) and only 3 species recorded. The dominant species in Haller Park were *Ae. tricholabis* (51.4%), *Ae. aegypti* (15.7%), and *An. funestus* (12.0%), while *Ae. aegypti* (85.1%) and *Ae. chausseri* (12.8%) predominated in Arabuko Sokoke.

The high species diversity and evenness distribution in Haller Park during the study despite missed short rains likely results from its mosaic of wetland patches, manmade pods, shaded vegetation, and anthropogenic influences, which together create multiple breeding niches. In contrast, the dense canopy and reduced sunlight penetration and reduced manmade pools in Arabuko Sokoke during dry season may limit larval development and reduce mosquito productivity (Karuitha et al., 2019; Wamae et al., 2010; Wang et al., 2016). Despite the lower abundance, the persistence

of *A e. aegypti* in forested habitats highlights its ecological plasticity and ability to exploit both sylvatic and peridomestic environments (Mangudo et al., 2015; Xia et al., 2020).

The coexistence of sylvatic and domestic *Aedes* species in forested habitats raises epidemiological concern, as these zones may serve as focal points for arbovirus maintenance and re-emergence through vector exchange between wildlife and humans (Diallo D. et al., 2012; Diallo D. et al., 2012; Xia et al., 2020).

Overall, the study shows a clear gradient in mosquito distribution and abundance: forested areas exhibited high species diversity but lower density, peri-urban areas supported both sylvatic and urban species, and urban areas recorded the highest overall abundance dominated by container-breeding mosquitoes. This pattern aligns with previous findings that vector species composition is influenced by habitat type, degree of urbanization, and human activity (Mwangangi et al., 2008; Ngugi et al., 2017).

5.2 Detection and Identification of Mosquito-Borne Arboviruses

The findings of this study underscore the continued circulation and ecological persistence of multiple arboviruses in mosquito populations from the Kenyan coastal region, particularly in forest–peri-urban interfaces where vectors diversity and host availability overlap.

Successful viral propagation in C6/36 cells, as evidenced by cytopathic effects in 50 of 200 pools, confirms the suitability of this mosquito-derived cell line for detecting arboviruses of medical importance. The characteristic rounding and detachment of infected C6/36 cells (Figure 12B) are consistent with viral infection by members of the *Flaviviridae* and *Togaviridae* families (Bolling et al., 2015; Vasilakis et al., 2009). The relatively low success rate in Vero cells suggests that mosquito-derived viruses may replicate more efficiently in insect cell lines than in mammalian ones, as previously observed in similar arbovirus surveillance studies in East Africa (Bolling et al., 2015).

These results highlight the importance of combining *in vitro* culture with molecular screening for comprehensive arbovirus surveillance, as not all viruses produce clear CPE or replicate equally across cell types.

RT-PCR assays targeting *Flavivirus* and *Alphavirus* genera detected 29 positive pools (23 Flavivirus, 6 Alphavirus). Spatially, Haller Park emerged as the primary hotspot for arbovirus detection, recording 16 (55.2%) flavivirus and 6 (20.7%) alphavirus-positive pools. This was followed by Bamburi with 5 (17.2%) flavivirus positives and Gede with 2 (6.9%) flavivirus positives. No virus was detected from Arabuko Sokoke or urban Mombasa sites. These findings indicate that virus circulation is highest in forested and peri-urban zones, likely due to the convergence of sylvatic and peridomestic mosquito populations and the presence of wildlife reservoirs (Diallo D. et al., 2012; Diallo D. et al., 2012).

The most significant vector associated with viral detection was *Aedes aegypti*, which accounted for the majority of positive pools across the study sites; 6 (27.27%) in Haller Park, 3 (60%) in Bamburi, and 2 (100%) in Gede. The detection of flavivirus RNA in *Ae. aegypti* confirms its well-established role as the principal vector of dengue, Zika, and yellow fever viruses in Kenya and globally (Agha et al., 2022; Ngugi et al., 2017). The detection of positive *Ae. aegypti* pools from both adult and larval stages suggests active vertical transmission, a phenomenon that sustains viral persistence during inter-epidemic periods (Mavale et al., 2010; Musa et al., 2020).

Other *Aedes* species also showed notable infection rates. *Ae. vittatus* and *Ae. tricholabis* each recorded four (13.8%) positive pools. *Ae. vittatus* was detected with both flaviviruses and alphaviruses, consistent with its known competence for multiple arboviruses, including Zika, chikungunya, and yellow fever (Diallo D. et al., 2012; Diallo D. et al., 2012). Similarly, *Ae. tricholabis*, typically a forest-associated mosquito, showed co-circulation of both virus types, highlighting the potential role of sylvatic vectors in maintaining arboviruses in forest ecosystems.

The co-detection of multiple viral genera in Haller Park indicates overlapping transmission cycles and possible interspecies virus exchange facilitated by mixed

vector populations and the presence of non-human primate reservoirs (Agha et al., 2017; Eastwood et al., 2020)

The spatial distribution of viral positivity suggests that forest and peri-urban zones serve as ecological bridges for arbovirus maintenance and emergence. The absence of viral detection in urban sites, despite high *Ae. aegypti* abundance, may reflect temporal variations in virus circulation or suppression through vector control activities. However, given the high vector densities in urban Mombasa, these environments remain at high risk of future arboviral outbreaks once viruses are introduced.

The dominance of *Ae. aegypti*, *Ae. vittatus*, and *Ae. tricholabis* among positive pools illustrates the importance of both domestic and sylvatic transmission cycles. The findings support the hypothesis that arboviruses persist in sylvatic mosquitoes and spill over to peri-urban populations during periods of environmental disturbance or increased human activity near forested zones (LaBeaud, et al., 2011; Musa et al., 2020).

This underscores the need for integrated surveillance strategies combining entomological, virological, and ecological monitoring to detect and respond to early signs of virus amplification before spillover into human populations. Vector control should focus on both peridomestic habitats and *forest edges*, where interaction between wild and domestic mosquito populations occurs.

5.3 Link between Sylvatic and Urban Transmission Cycles

The present study revealed a clear ecological gradient in mosquito species composition, abundance, and diversity across the forested, peri-urban, and urban zones of the Kenyan coastal region. The distinct community structures observed across these zones provide important insights into the ecological mechanisms that may facilitate the spillover and amplification of arboviruses between sylvatic and urban transmission cycles.

A total of 20 mosquito taxa were recorded, with species richness and diversity declining progressively from forested to peri-urban to urban sites. The forested sites (Haller Park and Arabuko Sokoke Forest) supported the highest diversity (Shannon index = 2.37), while the urban sites (Mvita, Kisauni/Nyali, Changamwe, and Likoni) recorded the lowest (Shannon index = 1.59). The evenness indices (0.53–0.58) also confirmed more balanced mosquito populations in natural habitats than in urbanized settings dominated by a few anthropophilic vectors.

In **urban areas**, *Aedes aegypti* and *Culex pipiens* overwhelmingly dominated, accounting for most of the specimens collected. These species are well-known to exploit man-made habitats such as tires, containers, and drainages, reflecting their high degree of ecological plasticity and adaptation to human-modified environments (Karuitha et al., 2019; Ngugi et al., 2017). Their predominance aligns with findings from other urban centers in East Africa where *Ae. aegypti* thrives as the principal vector of dengue, chikungunya, and Zika viruses (Ellis et al., 2015; Lutomiah et al., 2016)

Peri-urban zones, represented by Bamburi and Gede, supported a more mixed mosquito assemblage. While *Ae. aegypti* remained common, it co-occurred with *Anopheles funestus*, *Cx. pipiens*, and several *Aedes* species of sylvatic origin. This ecological overlap suggests transitional habitats where mosquitoes of both sylvatic and urban origin coexist, creating a potential interface for viral exchange between wildlife reservoirs and human populations.

In **forested zones** (Haller Park and Arabuko Sokoke), species diversity was highest, with *Aedes tricholabis* emerging as the dominant species, alongside *Ae. vittatus*, *Ae. simpsoni*, *Anopheles funestus*, and *Ficalbia mediolineata*. The diversity and balanced community structure (Simpson index = 0.69) indicate stable natural mosquito populations maintained by abundant breeding habitats and wildlife hosts typical of sylvatic ecosystems (Agha et al., 2017; Diallo D. et al., 2012; Eastwood et al., 2020).

The presence of *Ae. aegypti*, *Ae. tricholabis*, *Ae. vittatus*, and *Cx. pipiens* across all three ecological zones provides strong evidence for ecological connectivity between sylvatic and urban habitats. *Ae. aegypti* and *Ae. tricholabis* in particular were

recorded in both forested and peri-urban areas, indicating their capacity to bridge viral circulation between wildlife and human populations.

This ecological overlap supports the hypothesis of an active intermediate cycle, where peri-urban environments act as transition zones linking sylvatic and urban transmission systems. The adaptability of these vectors to both natural and artificial breeding sites allows them to maintain arboviruses in nature and facilitate periodic spillover to human populations (Gershman & Staples, 2016; Kraemer et al., 2015; Murillo et al., 2014).

Moreover, the detection of arboviruses (from Objective 2) in *Ae. aegypti* and *Ae. tricholabis*—species found across forest and peri-urban zones—provides virological evidence supporting the ecological link inferred from the species distribution data. This pattern is consistent with findings from Senegal where forest-dwelling *Aedes* species, including *Ae. furcifer* and *Ae. vittatus*, have been implicated in maintaining sylvatic cycles of yellow fever and chikungunya viruses that occasionally spill into human populations (Diallo D. et al., 2012, 2014; Diallo D. et al., 2012; M. Diallo et al., 2003).

The observed gradient in species diversity corresponds with the level of habitat disturbance. Forested areas, with minimal anthropogenic modification, harbour ecologically diverse mosquito communities with multiple potential arbovirus vectors. In contrast, urban environments, characterized by high human density and artificial breeding sites, support fewer species but higher vector abundance; particularly of *Ae. aegypti*, a competent arbovirus vector (Ngugi et al., 2017).

This pattern suggests that while arboviral diversity may originate and persist in sylvatic habitats, amplification and outbreak risk are greatest in urban centres where *Ae. aegypti* populations are dense and human–mosquito contact is frequent. Peri-urban zones, by hosting both sylvatic and anthropophilic species, serve as ecological corridors for virus introduction and adaptation to urban environments. The findings of this study highlight the critical role of peri-urban interfaces as bridges in arboviral ecology. The coexistence of vectors from multiple ecological origins enhances the potential for viral recombination, adaptation, and emergence of new strains capable

of urban transmission (Diallo D. et al., 2012; Eastwood et al., 2020) As such, surveillance and vector control efforts must not only target urban breeding sites but also extend to forest edges and peri-domestic zones where interspecies interactions are most intense.

Integrating ecological, entomological, and virological surveillance across habitat types is essential for detecting early signs of viral spillover and preventing large-scale transmission events. Conservation of natural ecosystems, coupled with sustainable urban planning, could also mitigate the disturbance-driven spread of arboviruses into human populations.

5.4 Host-Feeding Patterns, Phylogenetic Validation, and Vector–Host Connectivity

Objective Four sought to determine the host-feeding preferences of blood-fed mosquitoes and to evaluate their implications for arbovirus transmission and spillover risk along the sylvatic–urban gradient. The findings demonstrate that mosquito host use in the forested coastal ecosystem of Haller Park is strongly wildlife-oriented, with clear ecological links to peri-urban and urban transmission potential.

Molecular blood-meal analysis based on mitochondrial 12S gene sequences revealed that the majority of identified blood meals originated from wild mammalian hosts, including *Giraffa reticulata*, *Tragelaphus scriptus*, *Oryx beisa*, *Ichneumia albicauda*, and *Sylvilagus floridanus*. These results indicate active mosquito–wildlife interactions and support the presence of a stable sylvatic arbovirus transmission system within the study area. The predominance of *Aedes tricholabis* among blood-fed mosquitoes highlights its strong association with wildlife hosts and reinforces its ecological role in maintaining arboviruses within forested habitats.

Phylogenetic analysis using the Maximum Likelihood method further validated these host identifications. Blood-meal sequences clustered consistently with their respective vertebrate reference taxa, with high bootstrap support across major clades. Hosts detected at higher frequencies, particularly *Giraffa* spp. and *Tragelaphus*

scriptus, formed well-supported clusters (≥90%), despite moderate intraspecific sequence variation. Importantly, low-frequency and single-detection hosts also formed distinct, strongly supported lineages (≥96%), confirming the reliability of host assignments and the robustness of the molecular approach (Kumar et al., 2024). This phylogenetic concordance strengthens confidence in the observed host-feeding patterns and eliminates ambiguity in host resolution.

The bipartite mosquito–host interaction network provided additional insight into vector–host connectivity and feeding specialization. The network revealed a clear dominance of *Ae. tricholabis*, which was linked to multiple wildlife host species, indicating a generalist feeding strategy within sylvatic environments. In contrast, *Ae. aegypti* and *Cx. watti* exhibited limited host associations, reflecting either lower abundance or more restricted feeding behaviour within the forested setting. The central positioning of *Ae. tricholabis* within the network underscores its epidemiological importance as a potential bridge vector capable of linking multiple vertebrate hosts and facilitating virus persistence and transmission across ecological boundaries (Murillo et al., 2014; Musa et al., 2020).

The detection of *Ae. aegypti* feeding on wildlife, although infrequent, is epidemiologically significant. While *Ae. aegypti* is classically anthropophilic, its demonstrated feeding plasticity; particularly in forest-adjacent environments; suggests potential for viral exchange between sylvatic and domestic transmission cycles (Kamau et al., 2023; Scott & Takken, 2012). The coexistence of *Ae. tricholabis* and *Ae. aegypti* within Haller Park further highlights the risk of arbovirus amplification and adaptation at ecological interfaces, where wildlife, vectors, and humans increasingly overlap.

Although human-derived blood meals were not detected, the established mosquito–wildlife feeding network suggests that arboviruses can be maintained during inter-epidemic periods without human involvement. However, expanding human activity, tourism, and land-use change around Haller Park and neighbouring peri-urban areas such as Bamburi may increase contact rates, elevating spillover risk. The demonstrated connectivity between vectors and wildlife hosts therefore represents a

critical early warning signal for potential arboviral emergence (Kamau et al., 2023; Scott & Takken, 2012; Thiboutot et al., 2010).

In summary, the finding demonstrates that arbovirus transmission dynamics along the Kenyan coast are shaped by complex vector–host interactions operating within sylvatic systems but connected to peri-urban and urban environments. The integration of molecular blood-meal identification, phylogenetic validation, and bipartite network analysis provides strong evidence that *Ae. tricholabis* functions as a key sylvatic and potential bridge vector. These findings underscore the importance of incorporating host-feeding ecology and vector–host network structure into arbovirus surveillance and control strategies, particularly in ecological transition zones where spillover risk is highest.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

1. **Mosquito abundance and Diversity Vary Significantly Across Ecological Zones**

   Mosquito species composition and abundance differed markedly across forested, peri-urban, and urban habitats along the Kenyan coast, with key arbovirus vectors occurring across all zones, demonstrating ecological connectivity along the urban–forest continuum.

2. **Both natural and Artificial Larval Habitats Contribute to Vector Proliferation**

   A wide range of larval habitats, including artificial containers and natural water bodies, supported mosquito breeding, indicating that human-modified environments play a critical role in sustaining arbovirus vector populations.

3. **Active Circulation of Flaviviruses and Alphaviruses Occurs in Coastal Mosquito Populations**

   The molecular detection and isolation of arboviruses in mosquitoes confirm ongoing viral transmission, particularly in forest and peri-urban interface zones, highlighting these areas as hotspots for arboviral emergence.

4. **Bridge Vectors Facilitate Connectivity between Sylvatic and Urban Transmission Cycles**

   The overlapping distribution, host-feeding behaviour, and infection of *Aedes tricholabis* alongside *Aedes aegypti* indicate the potential role of peri-urban zones and bridge vectors in mediating arbovirus spillover from wildlife reservoirs to human populations.

6.2 Recommendations

1. **Implement Ecologically Stratified Vector Surveillance Programs** Continuous mosquito surveillance should be conducted across forest, peri-urban, and urban zones to capture spatial and seasonal variations in vector abundance and diversity and to enable early detection of shifts in transmission risk.
2. **Prioritize Larval Source Management Targeting Artificial Habitats**

   Vector control programs should focus on the removal of artificial containers, improvement of drainage systems, and better waste management, particularly in peri-urban and rapidly urbanizing areas where larval habitats are most abundant.

3. **Strengthen Integrated Arbovirus Surveillance in Ecological Transition Zones**

   Routine viral screening of mosquitoes should be intensified in forest–peri-urban interfaces such as Haller Park and Bamburi to enable early detection of flaviviruses and alphaviruses and to prevent spillover into urban populations.

4. **Adopt a One Health Approach to Arbovirus Prevention and Control**

   Future research and control strategies should integrate entomological, wildlife, livestock, and human serological surveillance to better understand transmission dynamics and to inform targeted, evidence-based interventions against arbovirus outbreaks.

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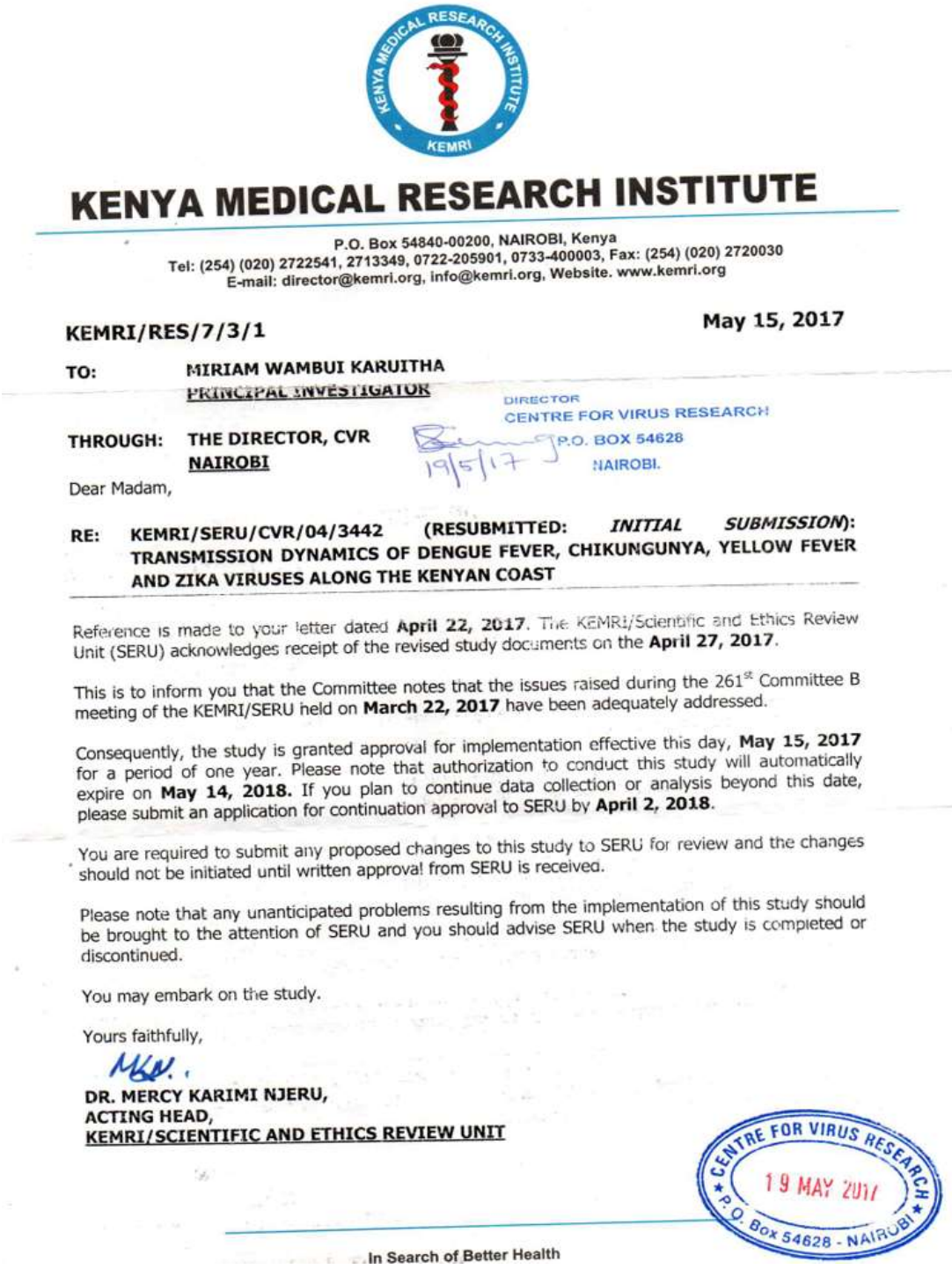
APPENDICES

Appendix I: KEMRI Ethical Approval



KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel: (254) (020) 2722541, 2713349, 0722-205901, 0733-400003, Fax: (254) (020) 2720030
E-mail: director@kemri.org, info@kemri.org, Website. www.kemri.org

KEMRI/RES/7/3/1 May 15, 2017

TO: MIRIAM WAMBUI KARUITHA
PRINCIPAL INVESTIGATOR

THROUGH: THE DIRECTOR, CVR
NAIROBI

DIRECTOR
CENTRE FOR VIRUS RESEARCH
P.O. BOX 54628
NAIROBI.
19/5/17

Dear Madam,

RE: KEMRI/SERU/CVR/04/3442 (RESUBMITTED: *INITIAL SUBMISSION*): TRANSMISSION DYNAMICS OF DENGUE FEVER, CHIKUNGUNYA, YELLOW FEVER AND ZIKA VIRUSES ALONG THE KENYAN COAST

Reference is made to your letter dated **April 22, 2017**. The KEMRI/Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised study documents on the **April 27, 2017**.

This is to inform you that the Committee notes that the issues raised during the 261st Committee B meeting of the KEMRI/SERU held on **March 22, 2017** have been adequately addressed.

Consequently, the study is granted approval for implementation effective this day, **May 15, 2017** for a period of one year. Please note that authorization to conduct this study will automatically expire on **May 14, 2018.** If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **April 2, 2018**.

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received.

Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued.

You may embark on the study.

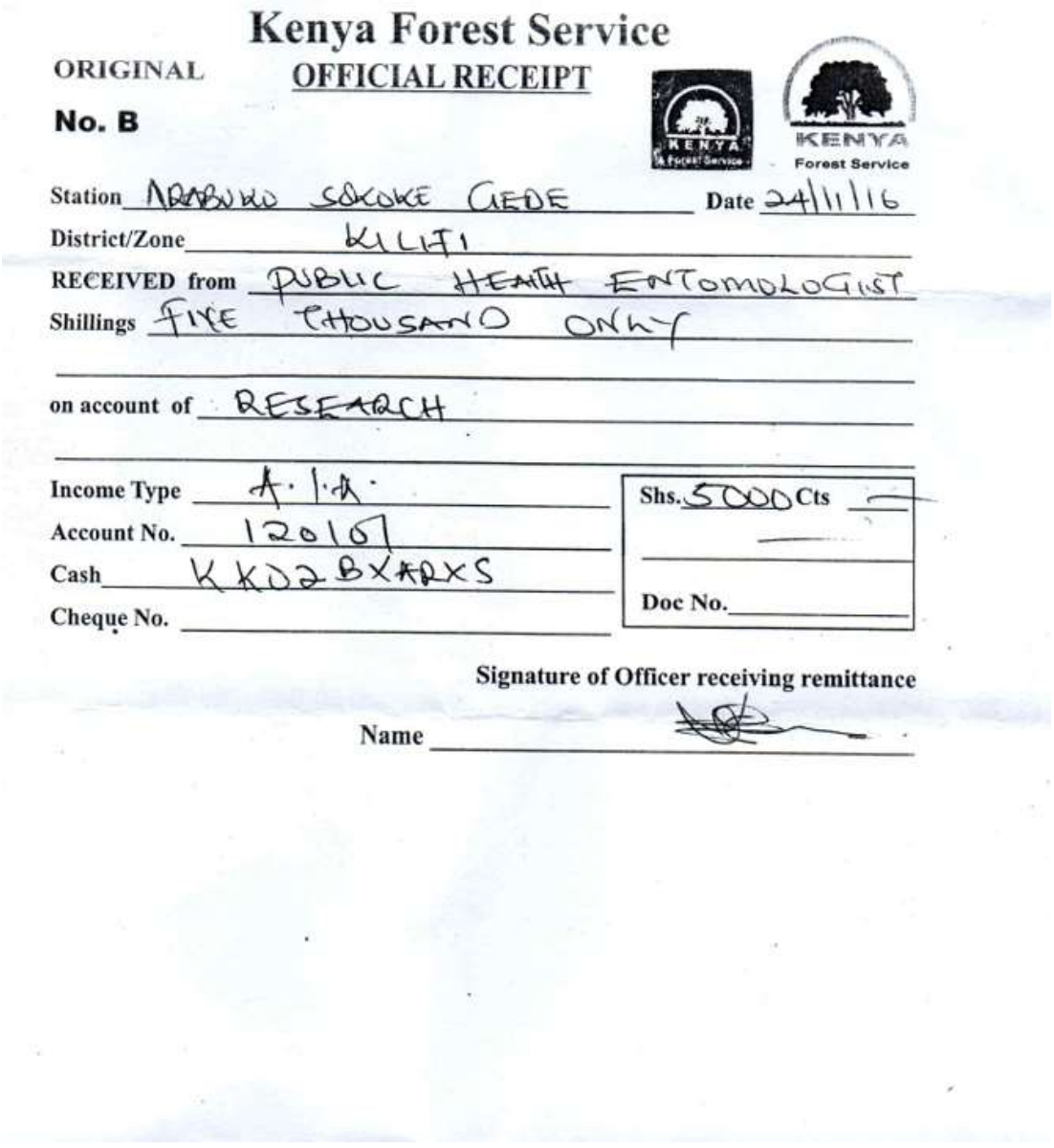
Yours faithfully,

DR. MERCY KARIMI NJERU,
ACTING HEAD,
KEMRI/SCIENTIFIC AND ETHICS REVIEW UNIT

CENTRE FOR VIRUS RESEARCH
19 MAY 2017
P.O. Box 54628 - NAIROBI

In Search of Better Health

Appendix II: Kenya Forest Service Official Receipt



Kenya Forest Service

ORIGINAL

OFFICIAL RECEIPT

No. B

KENYA Forest Service

Station NDABUKU SOKOKE GEDE Date 24/11/16

District/Zone KILIFI

RECEIVED from PUBLIC HEALTH ENTOMOLOGIST

Shillings FIVE THOUSAND ONLY

on account of RESEARCH

Income Type A.I.A.

Account No. 120107

Cash KKD2BXARXS

Cheque No.

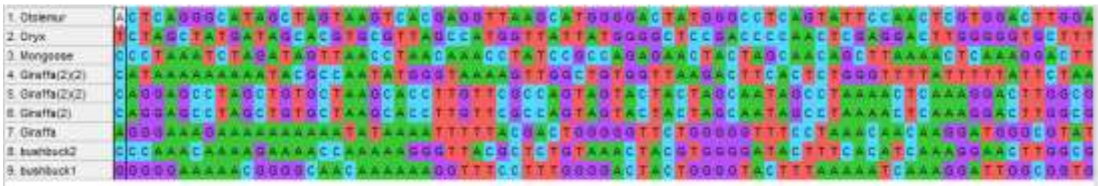
Shs. 5000 Cts —

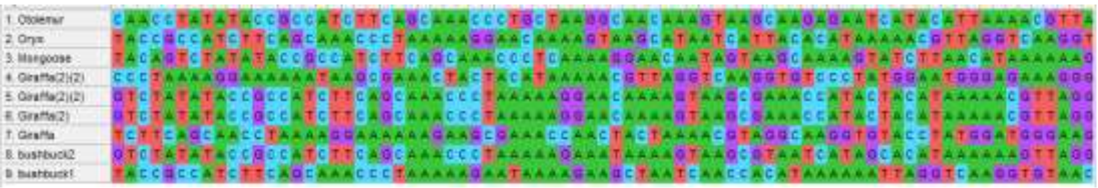
Doc No.

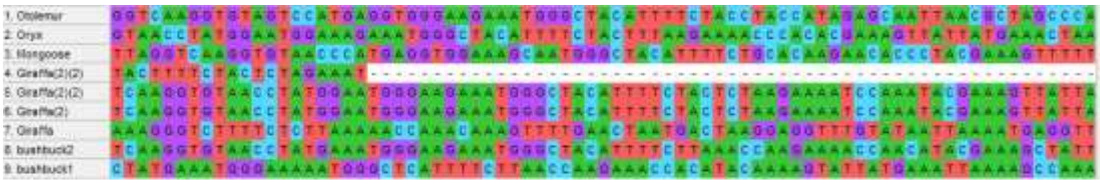
Signature of Officer receiving remittance

Name

Appendix III: Blood Sample Sequence Alignment







Score	Expect	Identities	Gaps	Strand
725 bits(392)	0.0	425/440(97%)	5/440(1%)	Plus/Plus

```
Query  39   AGAACTACTAGGCAACAGACTTAAAACTCAAAGGGACTTGGGCGGTGACTTCACATCCCT  98
Sbjct  560  ..........-.......-.............-....-.......-...........  614

Query  99   CTAGAGGAGCCTGTTCTATAATCGATAAACCCCGATAAACCTCACCACCTCTTGCTAATA  158
Sbjct  615  ..................................................C....  674

Query  159  CAGTCTATATACCGCCATCTTCAGCAAACCCTCAAAAGGAACAATAGTAAGCAAAAGTAT  218
Sbjct  675  ........................................T.G.............  734

Query  219  CTTAACATAAAAAAGTTAGGTCAAGGTGTAACCCATGAGGTGGAAAGCAATGGGCTACAT  278
Sbjct  735  ............................................................  794

Query  279  TTTCTGCACAAGAACACCCTACGAAAGTTTTTATGAAACCAAAAACTAAAGGAGGATTTA  338
Sbjct  795  .....A..........T.....................................  854

Query  339  GCAGTAAGCTGAGAACAGAGTGCTCAGCTGAATCGGGCCATGAAGCACGCACACACCGCC  398
Sbjct  855  ..............T.....................................  914

Query  399  CGTCACCCTCCTCAAGTATACAAATCTAAATATAACATATTAAAATAATACCCAATACAA  458
Sbjct  915  ..................C.........................C..C..T........  974

Query  459  GAGGAGACAAGTCGTAACAA  478
Sbjct  975  ....................  994
```

- P

  airwise alignment of sample 2 with the Subject/top hit *Ichneumia albicauda* mitochondrion, whole genome

Score	Expect	Identities	Gaps	Strand
364 bits(197)	4e-96	256/281(91%)	18/281(6%)	Plus/Plus

```
Query  54   TTAAAAATCAAAGGA-TTGGCGGTGCTTCCCTA--CCTCTA-AAGAACCTGTTCTATAAT  109
Sbjct  504  ......C........C.............-A..TC......G.G..G.............  562

Query  110  CG-TAAACCCCGA-AAACCTCACCAATTCTTGCTAATACAGTCTATATACCGCCATCTTC  167
Sbjct  563  ..A..........T.............................................  622

Query  168  AGCAAACCCTAAAAAGAA-TAAAAG-AAGC-TAATCA-ACCACATAAAAAA-TTAGGTCA  222
Sbjct  623  ..................A......T....G......T.G...........G........  682

Query  223  AGGTGTAACCTATGAAATGGGAA-AAATGGGCT-CATTTTCTTAA-CCAAGAAA-CCA-C  277
Sbjct  683  ......................G.........A...........A........A...A.  742

Query  278  ATACAAAAG-TATTATGAAATTAAAAGCCAAAGGAGGATTT  317
Sbjct  743  ....G....C.............T.................  783
```

*Sample 3 pairwise alignment with Top hit *Tragelaphus scriptus* from 12S mitochondrial ribosomal RNA gene

Score	Expect	Identities	Gaps	Strand
765 bits(414)	0.0	421/424(99%)	1/424(0%)	Plus/Plus

```
Query  62   TCAAAGGAACTTGGCGGTGCTTCATATCCCTCTAGAGGAGCCTGTTCTATAATCGATAAA  121
Sbjct  511  .......-..........................................................  569

Query  122  CCCCGATAAACCTCACCAATTCTTGCTAATACAGTCTATATACCGCCATCTTCAGCAAAC  181
Sbjct  570  ..........................................................  629

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Sbjct  630  ..........................................................  689

Query  242  ACCTATGAAATGGGAAGAAATGGGCTACATTTTCTTAAACCAAGAAAACCAACATACGAA  301
Sbjct  690  ..........................................................  749

Query  302  AGCTATTATGAAATTAATAGCCAAAGGAGGATTTAGCAGTAAACTAAGAATAGAGTGCTT  361
Sbjct  750  ..........................................................  809

Query  362  AGTTGAACCAGGCCATGAAGCACGCACACACCGCCCGTCACCCTCCTCAAACAATATTAA  421
Sbjct  810  ..........................................................  869

Query  422  TGCCCCAAAATTTATTTACATGCATTAACCACGAGAGAGGAGATAAGTCGTAACATAGTA  481
Sbjct  870  ..........................................................AG...  929

Query  482  AGCA  485
Sbjct  930  ....  933
```

Sample 4 pairwise alignment with Top hit *Tragelaphus scriptus* from 12S mitochondrial ribosomal RNA gene

Score	Expect	Identities	Gaps	Strand
747 bits(404)	0.0	417/423(99%)	1/423(0%)	Plus/Plus

```
Query  54   AACTC-GAGGACTTGGGGGTGCTTTATACCCTTCTAGAGGAGCCTGTTCTATAATCGATA  112
Sbjct  575  .....AA.........C.....................................------  634

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Sbjct  635  ..............................A.............................  694

Query  173  ACCCTAAAAAGGAACAAAAGTAAGCATAATCATTACACATAAAAACGTTAGGTCAAGGTG  232
Sbjct  695  ..........................................................  754

Query  233  TAACCTATGGAATGGAAAGAAATGGGCTACATTTTCTACTTTAAGAAAACCCACACGAAA  292
Sbjct  755  ..........................................................  814

Query  293  GTTATTATGAAACTAATAACCAAAGGAGGATTTAGTAGTAAGCTAAGAATAGAGTGCTTA  352
Sbjct  815  ..........................................................  874

Query  353  GCTGAATCAGGCCATGAAGCACGCACACACCGCCCGTCACCCTCCTCAAGTAATTTACGA  412
Sbjct  875  ..........................................................  934

Query  413  TGCACTTAAATTTATTACACGCACCAACCATATAAGAGGAGACAAGTCGTAACATGATAA  472
Sbjct  935  ..........................................................A.G...  994

Query  473  GCA  475
Sbjct  995  ...  997
```

*Sample 5alignned against top hit *Oryx beisa* isolate AWWP mitochondrial, complete genome

Score	Expect	Identities	Gaps	Strand
196 bits(106)	1e-45	170/196(87%)	23/196(11%)	Plus/Plus

```
Query  78   TCTAGA-GA-CCTGTTCT-T-ATCG-TAAACCCCGAT-TACCTC-CCAG-CCTTGCC-AT  128
Sbjct  608  ......G..G........A.A....A..........AA.....A....T.......A..  667

Query  129  ACAGT-TAT-TTCCGCC-TCTTCAGCAAACCCTAAAA-GGAA-AAAA-TAAGCGAAAC--  180
Sbjct  668  .....C...A.A.....A..................A....C....G..........CA  727

Query  181  TACTACATAAAAACGTTAGGTCAAGGTGTC-CCTATGGAATGGGA-GAAA-GGG-TAC-T  235
Sbjct  728  ............................AA.............A....T...C...A.  787

Query  236  TTTCTACTCTA-GAAA  250
Sbjct  788  ...........A....  803
```

*Sample7 aligned against top hit, giraffa tippekskirchi isolate GFOO7 mitochondirial ,complete genome

Score	Expect	Identities	Gaps	Strand
808 bits(437)	0.0	442/444(99%)	1/444(0%)	Plus/Plus

```
Query  26   TTGTTCGCCAGTAGTACTACTAGCAATAGCCTAAAACTCAAAGGACTTGGCGGTGCTTTA  85
Sbjct  543  ..A........-..................................................  601

Query  86   TATCCCTCTAGAGGAGCCTGTTCTATAATCGATAAACCCCGATAAACCTCACCAGTCCTT  145
Sbjct  602  ............................................................  661

Query  146  GCCAATACAGTCTATATACCGCCATCTTCAGCAAACCCTAAAAAGGAACAAAAGTAAGCG  205
Sbjct  662  ............................................................  721

Query  206  AAACCATACTACATAAAAACGTTAGGTCAAGGTGTAACCTATGGAATGGGAAGAAATGGG  265
Sbjct  722  ............................................................  781

Query  266  CTACATTTTCTACTCTAAGAAAATCCAAATACGAAAGTTATTATGAAACTAATGACTAAA  325
Sbjct  782  ............................................................  841

Query  326  GGAGGATTTAGTAGTAAACTAAGAATAGAGTGCTTAGTTGAATTAGGCCATGAAGCACGC  385
Sbjct  842  ............................................................  901

Query  386  ACACACCGCCCGTCACCCTCCTCAAATAGACACAATACATTCAAACTTATTAATACGTAT  445
Sbjct  902  ............................................................  961

Query  446  TAACCATGTGAGAGGAGATAAGTC  469
Sbjct  962  .........................  985
```

*Sample 10 aligned against top hit subject, *Giraffa camelopardalis camelopardalis* isolate GF088 mitochondrial, complete genome

Score	Expect	Identities	Gaps	Strand
808 bits(437)	0.0	442/444(99%)	1/444(0%)	Plus/Plus

```
Query  26   TTGTTCGCCAGTAGTACTACTAGCAATAGCCTAAAACTCAAAGGACTTGGCGGTGCTTTA  85
Sbjct  543  ..A........-..............................................  601

Query  86   TATCCCTCTAGAGGAGCCTGTTCTATAATCGATAAACCCCGATAAACCTCACCAGTCCTT  145
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Query  146  GCCAATACAGTCTATATACCGCCATCTTCAGCAAACCCTAAAAAGGAACAAAAGTAAGCG  205
Sbjct  662  ............................................................  721

Query  206  AAACCATACTACATAAAAACGTTAGGTCAAGGTGTAACCTATGGAATGGGAAGAAATGGG  265
Sbjct  722  ............................................................  781

Query  266  CTACATTTTCTACTCTAAGAAAATCCAAATACGAAAGTTATTATGAAACTAATGACTAAA  325
Sbjct  782  ............................................................  841

Query  326  GGAGGATTTAGTAGTAAACTAAGAATAGAGTGCTTAGTTGAATTAGGCCATGAAGCACGC  385
Sbjct  842  ............................................................  901

Query  386  ACACACCGCCCGTCACCCTCCTCAAATAGACACAATACATTCAAACTTATTAATACGTAT  445
Sbjct  902  ............................................................  961

Query  446  TAACCATGTGAGAGGAGATAAGTC  469
Sbjct  962  .........................  985
```

*Sample 11 aligned against top hit subject, *Giraffa camelopardalis camelopardalis* isolate GF088 mitochondrial, complete genome

Score	Expect	Identities	Gaps	Strand
361 bits(195)	1e-94	200/202(99%)	1/202(0%)	Plus/Plus

```
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Sbjct  576  ............................................................  635

Query  449  AACCCCGATAAACCTCACCAGTCCTTGCCAATACAGTCTATATACCGCCATCTTCAGCAA  508
Sbjct  636  ............................................................  695

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Sbjct  696  .C..........................................................  755

Query  569  T-ACCTATGGAATGGGAAGAAA  589
Sbjct  756  .A...................  777
```

*Sample 12 aligned against the subject *Giraffa tippelskirchi* isolate GF007 mitochondrial, complete genome

Score	Expect	Identities	Gaps	Strand
688 bits(372)	0.0	381/385(99%)	2/385(0%)	Plus/Plus

```
Query  95    TAAAGGAGCCTGTTCTATAATCGATAAACCCCGTATAAACCTCACCACCCCTTGCTAATT  154
Sbjct  629   ..G.............................-..........................  687

Query  155   CAACCTATATACCGCCATCTTCAGCAAACCCTGCTAAGGCAACAAAGTAAGCAAGAGAAT  214
Sbjct  688   ............................................................  747

Query  215   CATACATTAAAACGTTAGGTCAAGGTGTAGTCCATGAGGTGGGAAGAAATGGGCTACATT  274
Sbjct  748   ............................................................  807

Query  275   TTCTACCTACCATAGAGCAATTAACGCTAGCCCATATGAAACAATTGGCAAAAGGCGGAT  334
Sbjct  808   ................A...........................................  867

Query  335   TTAGTAGTAAATTAAGAATAGAGAGCTTAATTGAATAGGGCAATGAAGCACGCACACACC  394
Sbjct  868   ............................................................  927

Query  395   GCCCGTCACCCTCCTCAAAACTACTTTAGAACCTTAACATACATAATCCATACCACTAAA  454
Sbjct  928   ................-...........................................  986

Query  455   TTACTAGAGGAGATAAGTCGTAACA  479
Sbjct  987   .........................  1011
```

Sample 13 pairwise aligned against *Otolemur crassicaudatus* mitochondrion, complete genome

Appendix IV: Research Publication

Wellcome Open Research

Wellcome Open Research 2019, 4:175 Last updated: 23 MAR 2022

Check for updates

RESEARCH ARTICLE

Larval habitat diversity and mosquito species distribution along the coast of Kenya [version 1; peer review: 1 approved, 2 approved with reservations]

Miriam Karuitha[1,2], Joel Bargul[2,3], Joel Lutomiah[4], Simon Muriu[5], Joseph Nzovu[1], Rosemary Sang[4], Joseph Mwangangi[1,6], Charles Mbogo[1,7]

[1]Vector Biology Unit, Kenya Medical Research Institute (KEMRI), Center for Geographic Medicine Research Coast, Kilifi, P.O. Box 230-80100, Kenya
[2]Department of Biochemistry, Jomo Kenyatta University of Agriculture and Technology, Juja, Kenya
[3]The Animal Health Department, International Centre of Insect Physiology and Ecology, Nairobi, P.O. Box 30772-00100, Kenya
[4]Hemorrhagic Fever Unit, Kenya Medical Research Institute (KEMRI), Center for Virus Research, Nairobi, P.O. Box 62000-00200, Kenya
[5]Department of Biological Sciences, Pwani University Bioscience Centre (PUBREC), Kilifi, P.O Box 230-80100, Kenya
[6]Kenya Medical Research Institute (KEMRI), Center for Vector Disease Control, Kwale, Kenya
[7]KEMRI-Wellcome Trust Research Programme, Nairobi, P.O. Box 43640-00100, Kenya

v1 **First published:** 13 Nov 2019, **4**:175
https://doi.org/10.12688/wellcomeopenres.15550.1
Latest published: 13 Nov 2019, **4**:175
https://doi.org/10.12688/wellcomeopenres.15550.1

Abstract

Background: Management of arboviruses relies heavily on vector control. Implementation and sustenance of effective control measures requires regular surveillance of mosquito occurrences, species abundance and distribution. The current study evaluated larval habitat diversity and productivity, mosquito species diversity and distribution in selected sites along the coast of Kenya.
Methods: A cross-sectional survey of mosquito breeding habitats, species diversity and distribution was conducted in urban, peri-urban and forested ecological zones in Mombasa and Kilifi counties.
Results: A total of 13,009 immature mosquitoes were collected from 17 diverse aquatic habitats along the coast of Kenya. Larval productivity differed significantly ($F_{(16, 243)} = 3.21$, $P < 0.0001$) among the aquatic habitats, with tyre habitats recording the highest larval population. *Culex pipiens* (50.17%) and *Aedes aegypti* (38.73%) were the dominant mosquito species in urban areas, while *Ae. vittatus* (89%) was the dominant species in forested areas. In total, 4,735 adult mosquitoes belonging to 19 species were collected in Haller Park, Bamburi, Gede and Arabuko Sokoke forest. Urban areas supported higher densities of *Ae. aegypti* compared to peri-urban and forest areas, which, on the other hand, supported greater mosquito species diversity.
Conclusions: High *Ae. aegypti* production in urban and peri-urban areas present a greater risk of arbovirus outbreaks. Targeting

Open Peer Review

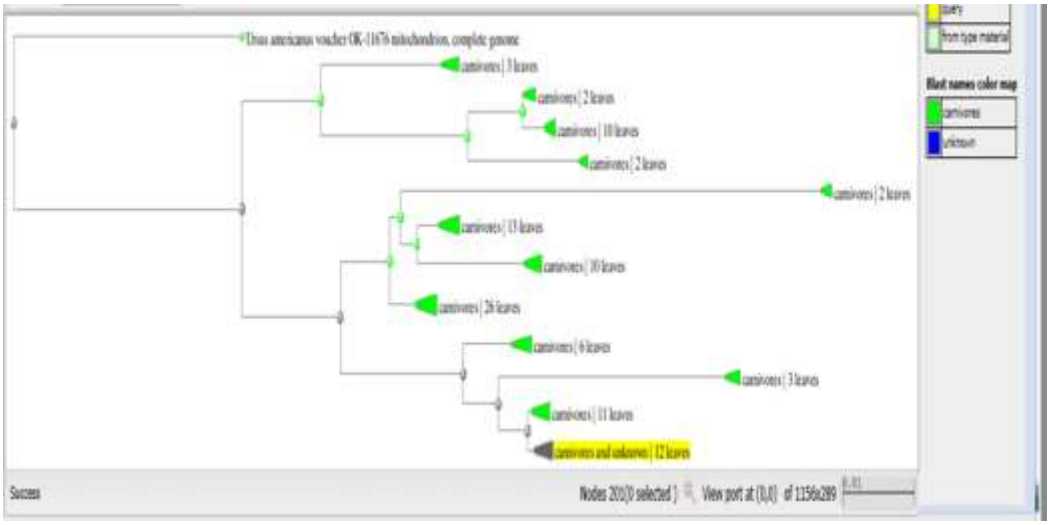
Approval Status ? ? ✓

	1	2	3
version 1 13 Nov 2019	? view	? view	✓ view

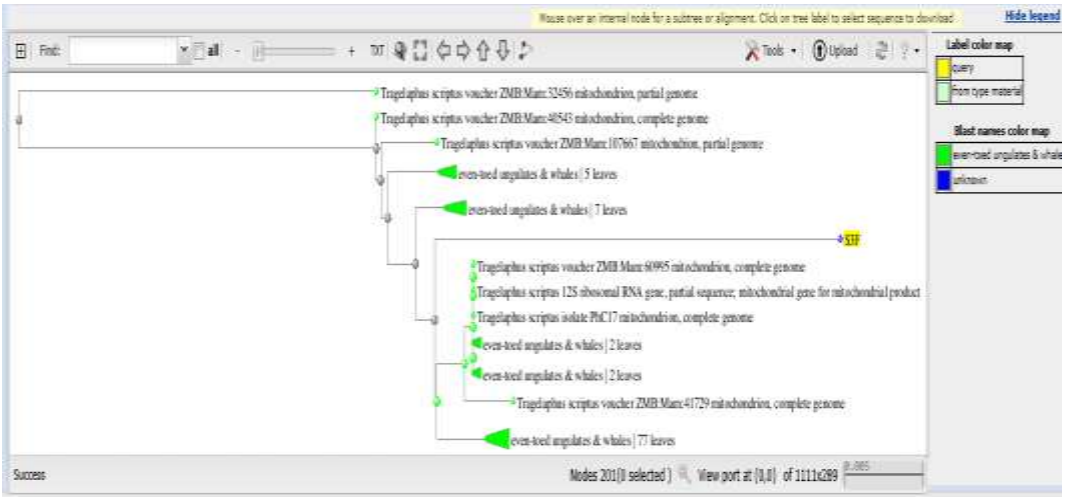
1. **Bryson Alberto Ndenga**, Kenya Medical Research Institute, Kisumu, Kenya
2. **John Paul Mutebi**, Centers for Disease Control and Prevention (CDC), Fort Collins, USA
3. **Francis M. Mutuku**, Technical University of Mombasa, Mombasa, Kenya

Any reports and responses or comments on the article can be found at the end of the article.

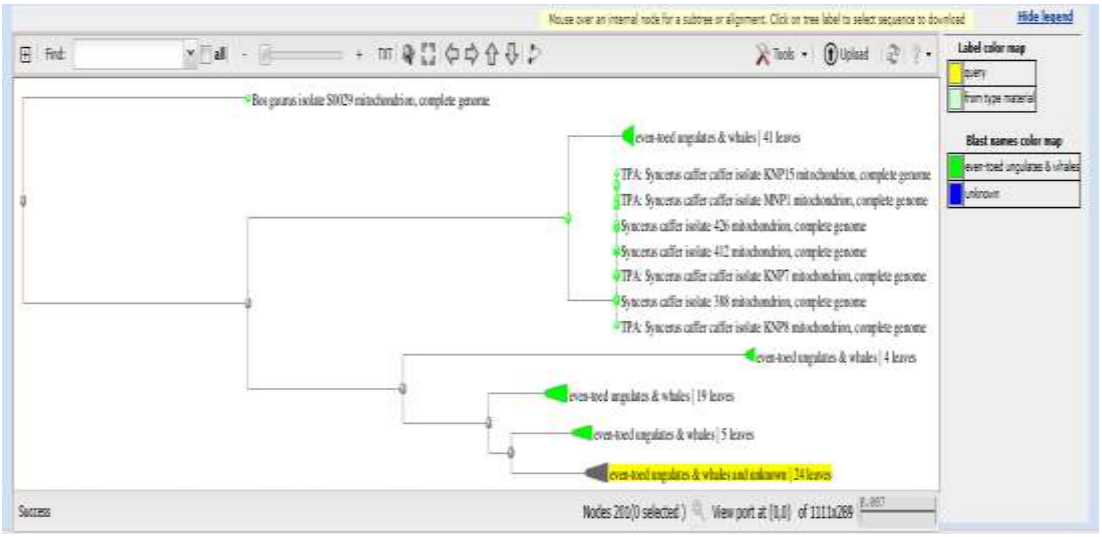
Appendices V: Supplementary Material



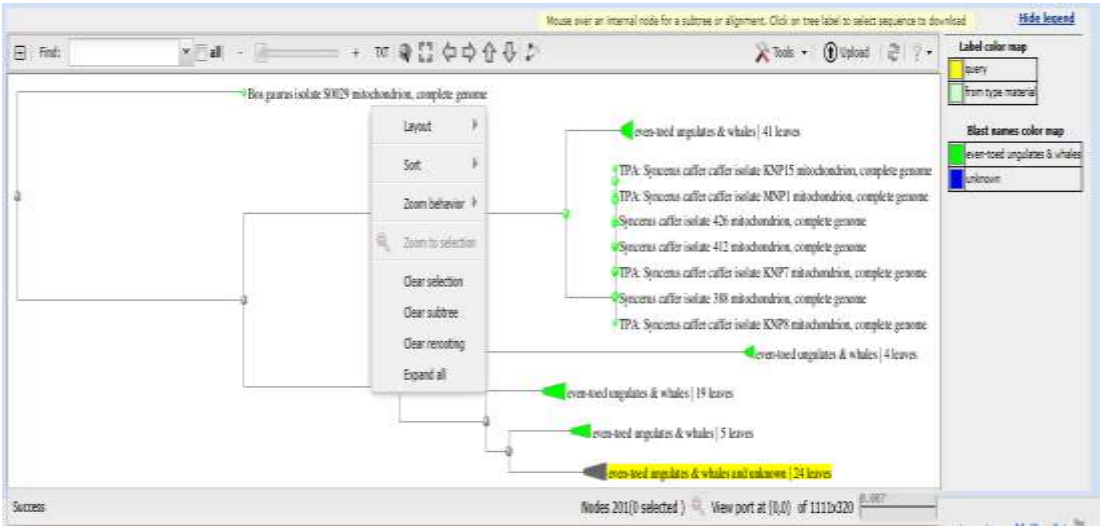
Sample 2: Mongoose



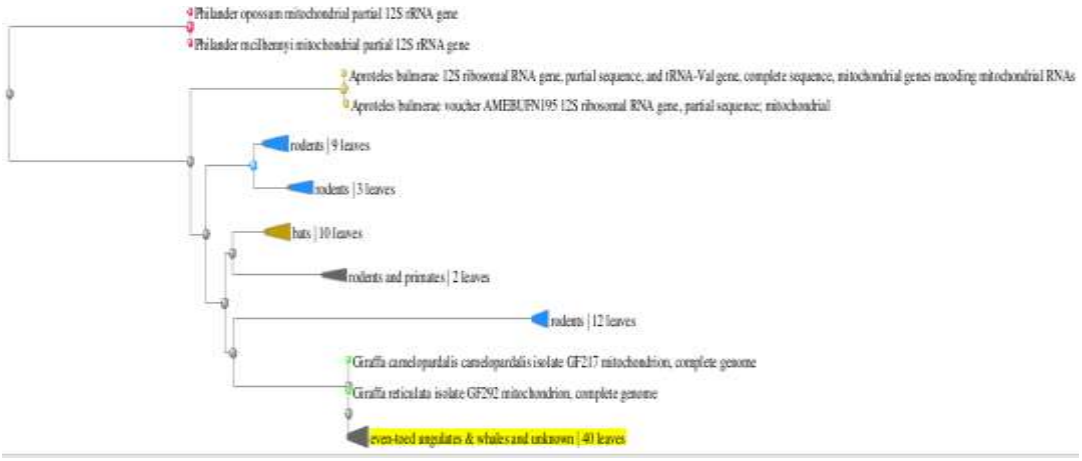
Sample 3: Bushback



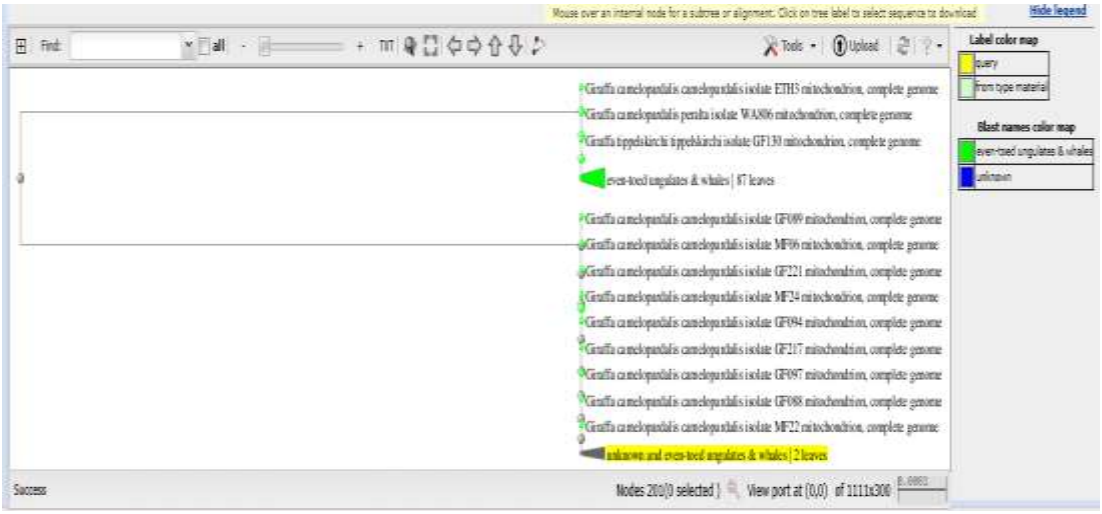
4: Bushback



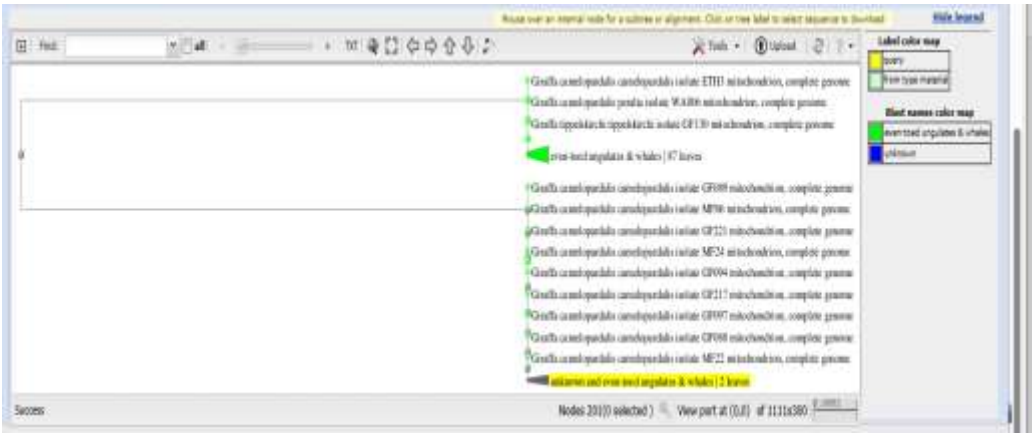
5: *Oryx beisa*



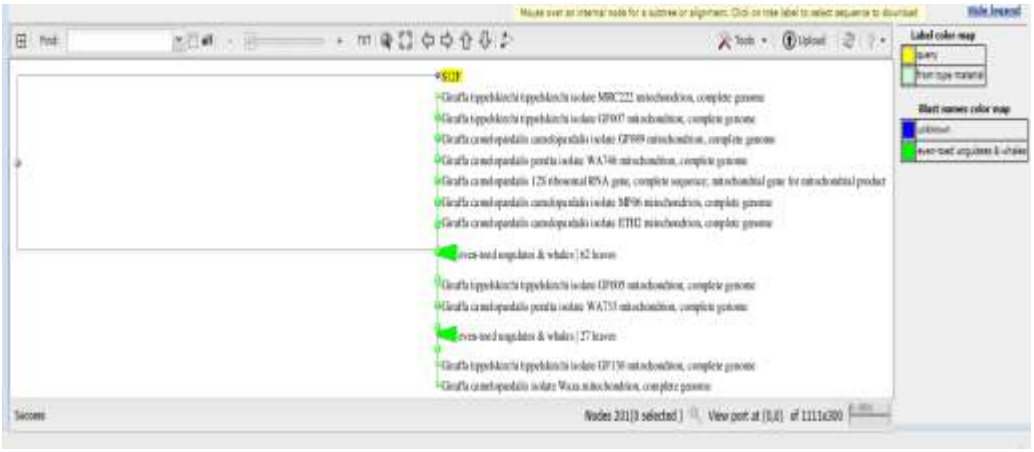
7: Giraffe



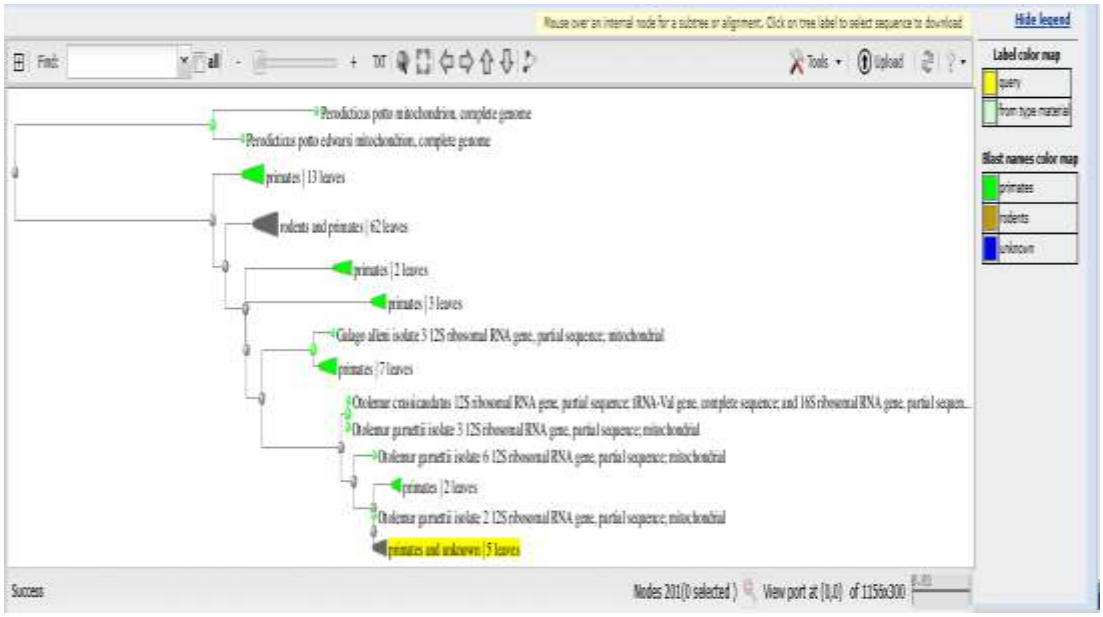
10: Giraffe



11: Giraffe



12: Giraffe



13: Brown great galago

*The phylogenetic trees show the placement of individual blood-meal sequences in relation to corresponding reference sequences of related vertebrate hosts retrieved from the GenBank database with the sample numbering 2,3,4,5,7,10,11,12 and 13(positive control).