YELLOW FEVER AND DENGUE VIRUS CIRCULATION AMONG HUMAN AND MOSQUITO POPULATIONS IN NGURUMAN AND KERIO VALLEY, KENYA

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Yellow Fever and Dengue Virus Circulation among Human and Mosquito Populations in Nguruman and Kerio Valley, Kenya

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DECLARATION

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

Signature…….Date…….

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DEDICATION

To my mother, Alice Boas, whose love and support have been my strength. Every step of this journey is a testament to your belief in me. I hope you are proud.

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Cynthia Chelangat and Titus Kitonyo: Your friendship and laughter kept me motivated and helped me endure countless hours in the lab.

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ABBREVIATIONS AND ACRONYMS

CCHFV	Crimean-Congo Haemorrhagic Fever Virus
CHIKV	Chikungunya Virus
CPE	Cytopathic Effect
DENV	Dengue Virus
DHF	Dengue Haemorrhagic Fever
DSS	Dengue Shock Syndrome
EYE	Eliminate Yellow Fever Epidemics
KEMRI	Kenya Medical Research Institute
ONNV	O'nyong-nyong virus
PRNT	Plaque Reduction Neutralization Test
RNA	Ribonucleic Acid
RT	Reverse transcriptase
RT-PCR	Reverse transcriptase – Polymerase Chain Reaction
WHO	World Health Organisation
WNV	West Nile virus
YFV	Yellow fever Virus
ZIKV	Zika Virus

MEGA	Molecular Evolutionary Genetics Analysis
CDC	Centre for Disease Control
DNA	Deoxyribonucleic Acid
DRC	Democratic Republic of Congo
FBS	Fetal Bovine Serum
DSS	Dengue Shock Syndrome
DHF	Dengue Hemorrhagic Fever

ABSTRACT

Dengue virus (DENV) and Yellow fever virus (YFV) are RNA viruses belonging to the genus *Flavivirus* and family *Flaviviridae*. Their emergence and re-emergence have become crucial public health problems, especially in Sub-Saharan Africa. Due to inadequate surveillance and diagnosis, prevalence and burden may be significantly underestimated. The main objective was to determine the circulation of DENV and YFV in mosquito and human populations in Kerio Valley and Nguruman, Kenya. 480 serum samples were collected from patients aged 5 to 85 years in a cross-sectional survey from July 2020 - May 2023 and tested for neutralizing antibodies against yellow fever virus and dengue viruses employing the Plaque Reduction Neutralization Test. In addition, 1822 pools of *Aedes* mosquitoes were collected and tested for DENV and YFV using cell culture and Reverse Transcriptase Polymerase Chain Reaction. A structural questionnaire was used to collect key demographic data. Using age, gender, and occupation as covariates, a multinomial logistic regression model was used to forecast risk for each of the most common viruses. Results indicated that, overall, neutralizing antibodies against at least one of the *Flaviviruses* analyzed were detected in 33.13%; 159/480 (95% CI, 50.1–59.0%) of the total samples, with larger proportions found in Kerio Valley (30.2%, 145/480) than in Nguruman (2.92%, 14/480) (P<0.0001). Generally, YFV had the highest seropositivity (32.5%) compared to DENV (0.63%). Dengue-neutralizing antibodies were detected only in Nguruman, where the seropositivity was low at 2%. This may be attributed to the dryland ecology in Nguruman, combined with climate change, which creates an ideal environment for Aedes mosquitoes, promoting DENV transmission. In Kerio Valley, women showed a significantly higher seroprevalence of neutralizing antibodies (60.82%) compared to men (p-value < 0.001), whereas in Nguruman, seropositivity rates were higher in men than in women at 47.47 per cent (P = 0.049). The study further shows that a diverse range of *Aedes stegomyia* mosquitoes exist in Nguruman and Kerio Valley with *Aedes aegypti* being the most predominant species (49.85%) followed by *Aedes metallicus* (16.5%), *Aedes simpsoni* (14.78%), *Aedes Chausseri* (2.37%) and *Aedes africanus* (1.7%). Out of the 1822 pools of mosquitoes tested, 2.8% (51/1822) showed cytopathic effects (CPE) on Vero cells (CCL-81 and E6). Nguruman had 3.35% of pools with CPE, while Kerio Valley had 1.94%. No significant difference was noted in viral activity between the two sites (p-value = 0.081, 95% CI). All 51 pools tested negative for *flavivirus* using the flavivirus universal primers (FU_1 and CFD_2). Further, upon sequencing, YFV and DENV viruses were not isolated from CPE-positive pools from both sites. The absence does not imply that these viruses are non-existent in these regions; rather, it may indicate low infection rates or viruses were not in circulation during the sampling period. The study concludes that yellow fever and dengue viruses are circulating among the human population in Nguruman and Kerio Valley, presenting a significant public health concern. Despite the presence of mosquito vectors, the viruses were not isolated from *Aedes* mosquitoes. The findings recommend strengthening surveillance of YFV and DENV in human and mosquito populations and using advanced methods like metagenomic testing for improved virus detection in mosquito from Nguruman and Kerio Valley.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Yellow fever virus and Dengue virus are members of the family *Flaviviridae* and genus *Flavivirus,* which are known to cause high rates of morbidity and mortality, particularly in sub-Saharan Africa (Chepkorir *et al.,* 2019; Inziani *et al.,* 2020; Torto & Tchouassi, 2024; World Health Organization, 2023). They are mostly endemic in subtropical and tropical regions (Agha *et al.,* 2017a; Mota *et al.,* 2021) where arthropod vectors are widespread. Both viruses are primarily spread by mosquitoes belonging to the *Aedes* subgenus *Stegomyia* (mainly *Aedes aegypti* and *Aedes albopictus*) and share an ecological niche with nonhuman primates acting as reservoir hosts (Sang *et al.,* 2022; Tajudeen *et al.,* 2022). In the last few decades, the severity and prevalence of these mosquito-borne arboviral diseases have significantly increased globally (Poinsignon *et al.,* 2025).

DENV is considered the fastest-spreading arbovirus globally (World Health Organization, 2023). The dengue virus exists in four closely related, but different serotypes namely DENV-1, DENV-2 , DENV-3 and DENV- 4 (Bosch *et al.,* 2020; Khan *et al.,* 2020), each of which causes dengue fever. About 390 million dengue cases are reported globally, with Africa accounting for 16% of all dengue cases (Agha *et al.,* 2019). An estimated half of the world's population is at risk due to dengue fever, which is currently found in more than 100 countries across Africa, the West Pacific, the Americas, Southeast Asia and Eastern Mediterranean (Bonney *et al.,* 2018; Torto & Tchouassi, 2024). Dengue virus is increasingly spreading into regions previously considered non-endemic, including parts of Europe such as France and Croatia, as well as countries like Afghanistan, due to expanding vector habitats facilitated by climate change and globalization (World Health Organization, 2023). In 2023, the highest number of dengue cases were recorded. There were 2300 fatalities and 4.5 million cases reported by the WHO Region of the Americas. Asia reported a significant number of cases, including 321 000 in Bangladesh, 111 400 in Malaysia, 150 000 in

Thailand, and 369 000 in Viet Nam (World Health Organization, 2023). A dengue fever infection can produce a range of signs and symptoms ranging from minor, vague signs to the hallmark dengue fever symptoms of high fevers and excruciating arthralgia (World Health Organization, 2023). DENV also causes dengue haemorrhagic fever, which can develop into lethal dengue shock syndrome (DSS) (Bonney *et al.*, 2018).

Dengue virus was discovered in Kenya's coastal region in Mombasa, Kilifi and Malindi in 1982 (Johnson *et al.,* 1982), followed by the 2011 outbreak in Mandera (Lutomiah *et al.,* 2016). Since then, subsequent dengue epidemics have been restricted to the Kenyan coast (Ellis *et al.,* 2015; Lutomiah *et al.,* 2016) and also recently affected the Somali-Kenyan border (Konongoi *et al.,* 2016). Kenya has reported cases of all three dengue viral serotypes (DENV 1-3), with DENV serotype 2 being the most prevalent (Konongoi *et al.,* 2016). Two Counties, Mandera and Mombasa, have reported the recent DENV outbreak in 2021 with a total of 2,359 cases and two fatalities being reported as of February 2022 (WHO, 2022). This geographic distribution of dengue epidemics is worrying because it shows the possibility of increased transmission in other areas (Agha & Tchouassi, 2022).

Yellow fever virus was first discovered in Kenya during the 1992-93 outbreak in Kerio Valley, Baringo County (Sanders *et al.,* 1998). Annual reports of YFV cases and deaths range from 30,000 to 200,000 respectively; the majority of these cases found in West and Sub-Saharan Africa (Agha *et al.,* 2017b). An estimated 33 countries, home to over 900 million people, are considered endemic regions for YFV where Africa accounts for approximately 90% of all global YFV infections (Garske *et al.,* 2014; Ramírez *et al.,* 2018). Nationwide, Kenya has reported 143 yellow fever cases and 12 deaths. The increase in the incidence of YFV outbreaks in Kenya is of great significance and requires immediate action. Moreover, Kenya has been classified as a high-risk country under the World Health Organization's Eliminate Yellow Fever Epidemics (EYE) Strategy (WHO, 2018). In addition, Isiolo County, reported 53 probable cases of yellow fever in March 2022, with six of those cases resulting in fatalities. Six ELISA-positive samples and two RT-PCR-positive samples suggested possible cases of yellow fever (WHO, 2022).

The rapid geographic spread of these arboviruses across Africa in recent decades is an alarming new trend, with severe outbreaks affecting urban human populations, in East and West Africa (Agha *et al.,* 2017b). An increase in these arboviral diseases has been connected to changes in the climate, environment, demographics, land-use patterns, and rising international travel (Egid *et al.,* 2022; Tigoi *et al.,* 2015). Roughly, 831 million individuals are in danger of contracting either one of these viruses in Africa. Because of the risk of global dissemination and the significant potential for devastating epidemics, mounting surveillance to monitor virus activity in diverse host populations and systems to identify transmission foci remains a top priority.

There is limited epidemiological and entomological data on the prevalence and distribution of YFV and DENV in Kerio Valley and Nguruman. The majority of resources for studying and controlling these viruses focus on epidemic periods, leaving a lack of surveillance capabilities (Chepkorir *et al.,* 2019). As a result, the level of exposure and the prevalence of these severe Flavivirus infections in these regions remain poorly understood (LaBeaud *et al.,* 2011). For instance, in 2020, the Kenya Medical Research Institute (KEMRI) performed laboratory tests that confirmed an outbreak of dengue fever in the Tiaty sub-county, Baringo. Laboratory-based Serological studies have also shown the presence of Dengue viruses in Kacheliba, West Pokot (Chepkorir *et al.,* 2019) and also in Mandera, Wajir and Garissa (Konongoi *et al.,* 2016; Langat *et al.,* 2020). This has confirmed DENV adaptation in dryland ecosystems. Nguruman and Kerio Valley having similar ecosystems to these areas necessitate the need for surveillance of these important viruses. Additionally, with the ongoing outbreak in Isiolo, several counties including Baringo County have been placed on high alert risk for yellow fever. Considering the recent recurrent yellow fever outbreaks in the neighbouring countries of Ethiopia, Uganda and South Sudan (Ahmed *et al.,* 2020; Chepkorir *et al.,* 2019; Demanou *et al.,* 2014; Lim *et al.,* 2020; Onyango *et al.,* 2004), as well as the risk of cross-border transmission through infected mosquitoes, non-human primates and humans, Kenya is also potentially in danger of Yellow Fever re-emergence. Several studies have also shown the presence of competent vectors. For instance, *Aedes africanus* and *Aedes keniensis* were collected from Baringo County where successful yellow fever virus has been detected (Johnson

et al., 1981; Karungu *et al.,* 2019; Reiter *et al.,* 1998). Moreover, *Aedes aegypti*, the principal dengue vector is also distributed widely in Kenya (Karungu *et al.,* 2019; Ngala & Chanasit, 2018; Ochieng *et al.,* 2013; Sang *et al.,* 2017). Thus, this research seeks to determine yellow Fever virus and dengue virus circulation among human and *Aedes* mosquitoes in Kerio Valley (Baringo County) and Nguruman (Kajiado County), Kenya.

1.2 Statement of the Problem

Yellow fever virus (YFV) and dengue virus (DENV) continue to pose a serious public health concern, with frequent outbreaks and serological evidence indicating continuous transmission (Chepkorir *et al.,* 2019; Ochieng *et al.,* 2015; Vu *et al.,* 2017). They are among the diseases that generate significant morbidity and mortality in endemic regions, imposing a growing burden on global and national health systems *(*Inziani *et al.,* 2020). Their impact is often underestimated in tropical and subtropical areas. In Kenya, both viruses are common, with data from serological research and mosquito isolation studies (Tigoi *et al.,* 2015; Konongoi *et al.,* 2016; Agha *et al.,* 2017; Atoni *et al.,* 2018; Chepkorir *et al.,* 2019; Inziani *et al.,* 2020) showing a wide distribution of arboviruses throughout Kenya, thus suggesting a potential concern to public health. The lack of reliable burden data limits the ability of health authorities to allocate resources, plan effective interventions, or respond swiftly to outbreaks.

The incidence of dengue in Kenya has risen sharply in recent years. Outbreaks have been reported in Mombasa and Mandera, and studies show ongoing transmission in inland regions (Vu *et al.,* 2017). Yellow fever is less frequently reported but still poses a risk. The outbreak in Kerio Valley from 1992 to 1993 resulted in several hundred cases and deaths (WHO, 2022). Due to asymptomatic or mild infections, poor surveillance systems, and insufficient monitoring of mosquito populations, the true burden of these diseases is likely underreported (LaBeaud *et al.,* 2011; Shah *et al.,* 2020).

Although there is a safe and effective vaccine against YFV, the vaccine is not easily available and the prices are high for the general population. There is a novel, partially

authorized vaccine for DENV, but is only for use in high disease-burden areas. Yet, yellow fever epidemic is still a risk in Kenya because routine YFV vaccination coverage is estimated to be very low (7%) and vaccinations are only limited to four counties in the country's west (Baringo, Elgeyo Marakwet, West Pokot and Turkana) (WHO, 2022).

Furthermore, diagnosis of these arboviruses is frequently hindered by clinical manifestations which mimic other common fever-causing illnesses like typhoid fever and malaria, as well as the lack of diagnostic tools in the majority of health facilities (Konongoi *et al.*, 2016); hence their prevalence is underappreciated in Kenya. There is also the presence of YFV and DENV vectors in Kerio Valley and Nguruman which portrays the potential for the emergence of these diseases (Karungu *et al.*, 2019).

In addition to limited vaccine access and diagnostic issues, several other challenges make prevention and control difficult. These challenges include insecticide resistance in mosquitoes, inadequate waste and water management in expanding urban areas, climate change effects on mosquito habitats, and low public awareness of the symptoms and prevention methods (Tajudeen *et al.*, 2022). The absence of region-specific data on mosquito infection rates and human exposure further limits Kenya's ability to anticipate outbreaks and effectively target control efforts.

1.3 Justification of the Study

Dengue virus and yellow fever virus have been implicated in major outbreaks in Kenya. However, the lack of adequate surveillance and assessment of risk for their occurrence and re-emergence, particularly in ecologically diverse and remote regions such as Kerio Valley and Nguruman is concerning (Sang *et al.*, 2022). Nguruman and Kerio Valley are ecologically diverse areas characterized by interactions between humans, wildlife, vectors, and limited health surveillance, which create favourable conditions for the emergence and persistence of arboviruses. The yellow fever outbreak that occurred in 1992/93 in Kerio Valley highlighted the region's vulnerability; yet, limited seroprevalence studies have been conducted since. Additionally, dengue virus has emerged in inland counties such as Mandera and

Garissa, which prompts concerns about its potential spread to ecologically similar but surveillance-poor regions, like Nguruman and the Kerio Valley (Vu *et al.*, 2017). Evidence of DENV and YFV antibodies in non-outbreak areas suggests silent or underreported transmission, possibly driven by sylvatic or peri-domestic cycles (Chepkorir *et al.*, 2019; Kibathi *et al.*, 2024).

Dengue virus and yellow fever virus share the same vectors (mostly *Aedes* mosquitoes), hosts, and ecological niche in the ecosystem. Hence, it is very beneficial to study both viruses at the same time since combined surveillance and control methods can deepen the knowledge of the dynamics of arboviral transmission in a shared ecosystem. Therefore, it is essential to conduct surveillance to monitor disease circulation and assess risk to human populations to institute early detection and appropriate control strategies. This study informs tailored control efforts on areas with proven risk, saving the country much-needed scarce resources. In addition, risk information informs targeted control measures, i.e. vaccination and vector population control.

1.4 Research Questions

1) What is the seroprevalence of yellow fever virus and dengue virus in human samples from Kerio Valley and Nguruman, Kenya?
2) What is the viral activity in *Aedes Stegomyia* mosquitoes collected from Nguruman and Kerio Valley, Kenya?
3) What are the circulating genotypes and serotypes of yellow fever virus and dengue virus isolated from *Aedes Stegomyia* mosquitoes from Nguruman and Kerio Valley, Kenya?

1.5 Objectives

1.5.1 General Objective

To determine the distribution of yellow fever and dengue virus among human and mosquito populations in Nguruman and Kerio Valley, Kenya.

1.5.2 Specific Objectives

1) To determine the seroprevalence of yellow fever and dengue viruses in human samples from Kerio Valley and Nguruman, Kenya.
2) To demonstrate viral activity in *Aedes Stegomyia* mosquitoes collected from Kerio Valley and Nguruman, Kenya.
3) To identify the circulating genotypes and serotypes of yellow fever and dengue viruses isolated from *Aedes Stegomyia* mosquitoes from Nguruman and Kerio Valley.

CHAPTER TWO

LITERATURE REVIEW

2.1 Arboviruses

Dengue and yellow fever are arboviruses transmitted biologically by way of hematophagous arthropod vectors, notably mosquitoes, sand flies, and ticks, which feed on the blood of their susceptible vertebrate hosts (Adam & Jassoy, 2021; Uwishema *et al.,* 2022). *Flaviviridae, Togaviridae, Bunyaviridae,* and *Reoviridae* are important families within the arboviruses (Calisher *et al.,* 2019). In these families, dengue virus (DENV), yellow fever virus (YFV), West Nile virus (WNV), Zika virus (ZIKV), (Flaviviridae), O'nyong-nyong virus (ONNV), chikungunya virus (CHIKV), (Togaviridae), Crimean–Congo haemorrhagic fever virus (CCHFV) (Bunyaviridae) and Rift Valley fever virus (RVFV) are medically significant because of the prevalence, severity, and sporadic outbreaks of diseases they cause (Adam & Jassoy, 2021; Silva *et al.,* 2020). These arboviruses circulate among wild animals in sylvan, rural, urban, and peri-urban environments, where they spread to people and/or domestic animals who serve as incidental or terminal hosts, resulting in significant illness and mortality (Huang *et al.,* 2014; Mease *et al.,* 2011; Ramírez *et al.,* 2018). The vectors become infected after ingesting an infectious blood meal. The virus multiplies in the tissues of the vector during this extrinsic incubation period, and then it spreads to vulnerable vertebrate hosts through salivary secretions during successive meals, causing viremia and occasionally sickness (Wazir *et al.,* 2024) For the majority of these viruses, no specific treatments or vaccines are available for the prevention and management of infections, despite their significant impact on public health and the potential for epidemics (Nanaware *et al.,* 2021; Wazir *et al.,* 2024). Thus, understanding the likelihood of transmission and the potential of the related vectors provides crucial knowledge that can be used to avoid and/or control epidemics with precision *(Agha et al.,* 2017a; Chepkorir *et al.,* 2019).

2.2 Family Flaviviridae

The genera Pegivirus, *Flavivirus*, *Hepacivirus* and *Pestivirus*, are all members of the family *Flaviviridae*. There are 53 virus species in the genus *Flavivirus* (Chepkorir, 2019; Simmonds *et al.*, 2017). Several species of mosquito, as well as argasid and ixodid ticks, transmit arboviruses in the genus Flavivirus (Azar & Weaver, 2019; Diallo *et al.*, 2014; Lwande *et al.*, 2013; Ochieng *et al.*, 2013; Pfeffer & Dobler, 2010). However, in this study, the emphasis is on four *Flaviviruses* previously found to be present in East Africa: West Nile virus (WNV), yellow fever virus (YFV), dengue virus (DENV), and Zika virus (ZIKV) (Chepkorir *et al.*, 2019). The necessity to concentrate on these viruses to avoid cross-reactivity steming from reports that *flavivirus* antibodies exhibit cross-reactivity with other closely related *flaviviruses*. This family of viruses has a fairly wide geographic distribution, and like other arboviruses, each virus's distribution reflects vector distribution (Huang *et al.*, 2014).

2.2.1 Genome Organisation and Replication Cycle of Yellow Fever Virus and Dengue Virus

Yellow fever and dengue viruses are related and remarkably similar to one another. They both belong to the genus *Flavivirus* and *Flaviviridae* family. Flaviviruses are single-stranded, positive-sense RNA viruses characterized by an enveloped icosahedral capsid and approximately 11 kb genome (Pierson & Diamond, 2020). The genome has a single-long open reading frame (Ciota & Kramer, 2010). The *Flavivirus* genome translates into a single polyprotein that is cleaved into three structural proteins (Capsid (C), precursor Membrane (prM), and Envelope (E)) and seven non-structural proteins namely NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 which help in replication (Nanaware *et al.*, 2021; Pierson & Diamond, 2020). The genome is organized in the same organisation from 5′ –C – prM – M – E – NS1 – NS2A – NS2B – NS3 – NS4A – 2K – NS4B – NS5 – 3′ (Chepkorir, 2019; Onyango *et al.*, 2004) (Figure 2.1 and 2.2A). Viral entry is facilitated through receptor-mediated endocytosis, where the low pH environment within the endosome induces conformational changes in the envelope (E) protein, promoting fusion between the viral envelope and the endosomal membrane. Following fusion, the viral genome is released into the

cytoplasm, where replication takes place on modified endoplasmic reticulum membranes where it is utilized by the RNA-dependent RNA polymerase (RdRp) to drive both translation and replication processes. The viral RNA serves both as a template for translation and as a substrate for RNA replication, producing a negative-sense intermediate used to synthesize new genomic RNA (Nanaware *et al.*, 2021). Newly synthesized viral proteins and RNA genomes assemble into progeny virions, which acquire their envelope from the host cell membrane. Viral assembly occurs in the endoplasmic reticulum, followed by maturation in the Golgi apparatus and release via exocytosis enabling them to infect neighbouring cells and propagate the infection (Chepkorir, 2019) (Figure 2.1 and 2.2). This tightly regulated replication cycle enables efficient viral propagation and evasion of the immune system. Notably, both DENV and YFV exhibit tropism for hepatic and dendritic cells, contributing to their pathogenesis, which ranges from mild febrile illness to severe hemorrhagic manifestations and organ failure

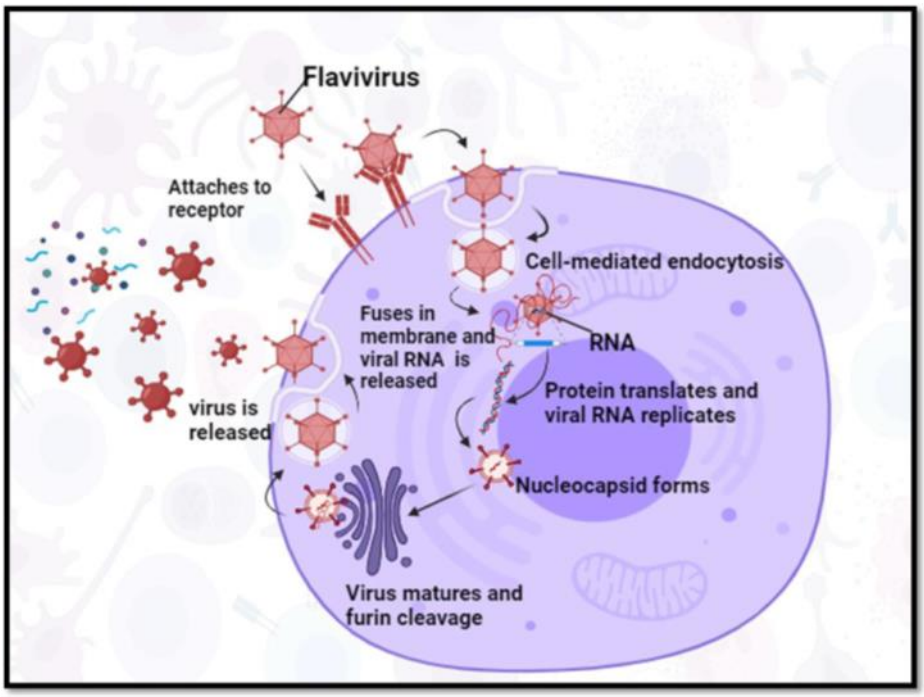


Figure 2.1: Replication Cycle of Yellow Fever Virus (YFV)

Source:(Wazir *et al.*, 2024)

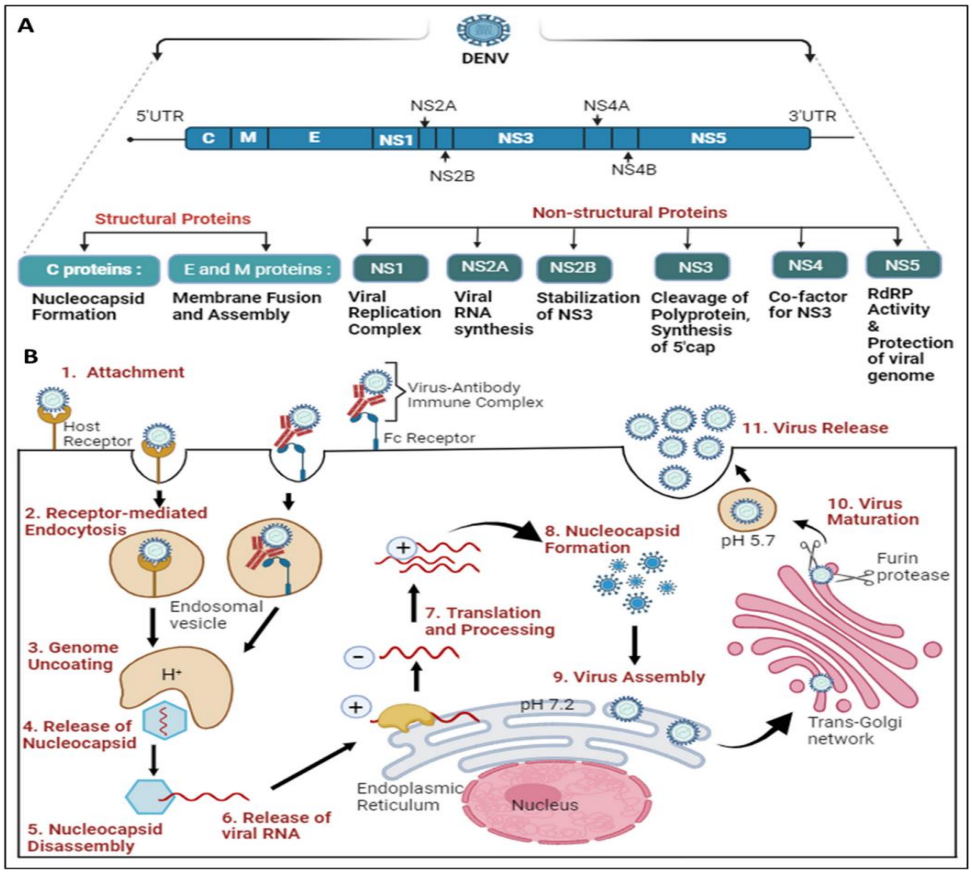


Figure 2.2: Diagram of (A) The Dengue Virus Genome Structure and (B) Dengue Virus Replication

Source: (Nanaware *et al.*, 2021; Sinha *et al.*, 2024)

2.3 Pathogenesis and Clinical Presentations of Dengue and Yellow Fever

The pathogenesis of both dengue virus (DENV) and yellow fever virus (YFV) involves the complex interactions between the virus, host immune response, and target cells. Dengue virus pathogenesis begins after the bite of an infected Aedes mosquito (Sang *et al.*, 2022) , where the virus targets dendritic cells, monocytes, and macrophages at the site of inoculation. These infected cells transport the virus to lymphoid tissues, enabling systemic dissemination and viremia. The dengue virus results in both primary and secondary infections (Mease *et al.*, 2011). The primary infection is an acute feverish sickness called Dengue Fever (DF), which often is cleared within a week (7 days) by the immune system and is characterised by sudden-onset high fever, retro-

orbital pain, myalgia, arthralgia, rash, and headache(Agha & Tchouassi, 2022; Chepkorir, 2019). The secondary infections (Dengue Shock Syndrome (DSS) or Dengue Haemorrhagic Fever (DHF)) are more severe and can lead to dengue infection-related fatalities associated with plasma leakage, bleeding tendencies, and organ impairment (Abdullah *et al.,* 2020; Konongoi *et al.,* 2016). A critical driver of severity is antibody-dependent enhancement (ADE), wherein non-neutralizing antibodies from a previous infection enhance viral uptake via Fcγ receptors, amplifying viral replication and inducing a cytokine storm. This immune dysregulation increases vascular permeability, leading to hypovolemia and shock, particularly in secondary infections (Pourzangiabadi *et al.,* 2025).

Yellow fever virus (YFV), on the other hand, follows a different pathogenic course. After local replication, the virus spreads to lymph nodes and then to visceral organs, with a notable hepatotropism. Infected hepatocytes undergo apoptosis and necrosis, forming councilman bodies, a histological hallmark of yellow fever. Clinically, YFV infection presents after an incubation period of 3–6 days with non-specific symptoms such as fever, chills, headache, nausea, and myalgia (Mease *et al.,* 2011). This initial phase may resolve, but in 15–25% of cases, patients progress to a toxic phase marked by jaundice, hemorrhagic manifestations (e.g., epistaxis, hematemesis), renal dysfunction, and shock. Unlike dengue, yellow fever lacks immune enhancement mechanisms like ADE; however, severe disease correlates with high viremia and impaired innate immune responses. Both YFV and DENV share arthropod-borne transmission and febrile presentations, but differ markedly in their pathophysiological processes and organ-specific manifestations (Pourzangiabadi *et al.,* 2025).

2.4 Transmission Cycle for Yellow Fever Virus and Dengue Virus

The primary method of transmission for DENV and YFV is via the bite of an infected female *Aedes* mosquito of the subgenus *Stegomyia* (Huang, 2004; Monath, 2021). Although DENV's precise origin is unknown, YFV originated in Africa (Onyango *et al.,* 2004). Both viruses have a history of developing into persistent human carriers through *Aedes aegypti*. They both began in sylvatic cycles and are maintained in forest-dwelling *Aedes* mosquitoes and non-human primates (Hanley *et al.,* 2013).

Transmission of YFV follows three recognised ecological cycles: sylvatic (jungle), intermediate (savannah), and urban, as described in Figure 2.3. Nonhuman primates and tree-hole breeding mosquitoes are involved in the primary transmission cycle (sylvatic). Human involvement defines the urban cycle; exposure to infected mosquitoes leads to human infection. In Africa, YFV viral cycles are sylvatic. *Aedes furcifer, Aedes africanus, Aedes opok, Aedes taylori and Aedes luteocephalus* are the sylvatic vectors in Africa (Diallo *et al.,* 2003; Srivastava *et al.,* 2024) while in the Americas, sylvatic transmission has been attributed to *Haemagogus* species such as *leucocelaenus*, *albomaculatus*, *spegazzini*, and *janthinomys*, as well as *Sabethes* species including *chloropterus*, *albipivus*, *glaucodaemon*, *soperi*, and *cyaneus* (Srivastava *et al.,* 2024). The intermediate (savannah) cycle, primarily found in parts of Africa, involves transmission between humans and mosquitoes in rural or semi-urban settings, often during outbreaks. In this cycle, *Aedes* species that breed around human dwellings play a key role. The urban transmission cycle occurs when infected individuals introduce the virus into densely populated areas with high *Aedes aegypti* populations. In such environments, human-to-human transmission is sustained by mosquito bites, leading to rapid spread and potentially large outbreaks. These overlapping cycles highlight the ecological complexity and public health risk posed by the yellow fever virus.

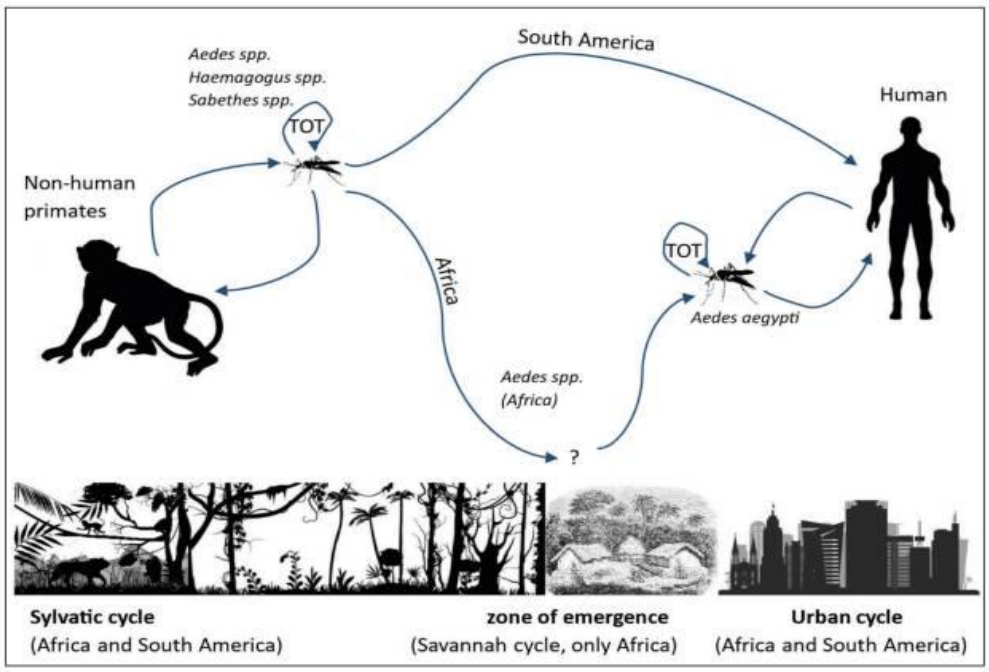


Figure 2.3: Schematic Representation of the Yellow Fever Virus Transmission Cycle, Illustrating the Sylvatic (Jungle), Intermediate (Savannah), and Urban Cycles

Source:(Srivastava *et al.*, 2024)

Dengue virus transmission follows an urban cycle involving humans and mosquitoes of the *Aedes* genus, especially *Aedes aegypti* and *Aedes albopictus* (Agha & Tchouassi, 2022; Sang *et al.*, 2022). The virus is sustained in this cycle by human-mosquito-human transmission. A female mosquito contracts the virus when it feeds on an infected person's blood during the viremic phase. The virus multiplies and enters the salivary glands of the mosquito after an extrinsic incubation period, allowing the mosquito to infect new people through bites (Wazir *et al.*, 2024). In addition to the well-known urban cycle, a sylvatic (enzootic) transmission cycle exists in parts of Africa and Southeast Asia. In this cycle, DENV circulates between non-human primates and forest-dwelling *Aedes* mosquitoes, with humans occasionally infected through spill over when they encroach into forested habitats (Sang *et al.*, 2022). The transmission cycle of the dengue virus is shown in Figure 2.4 below.

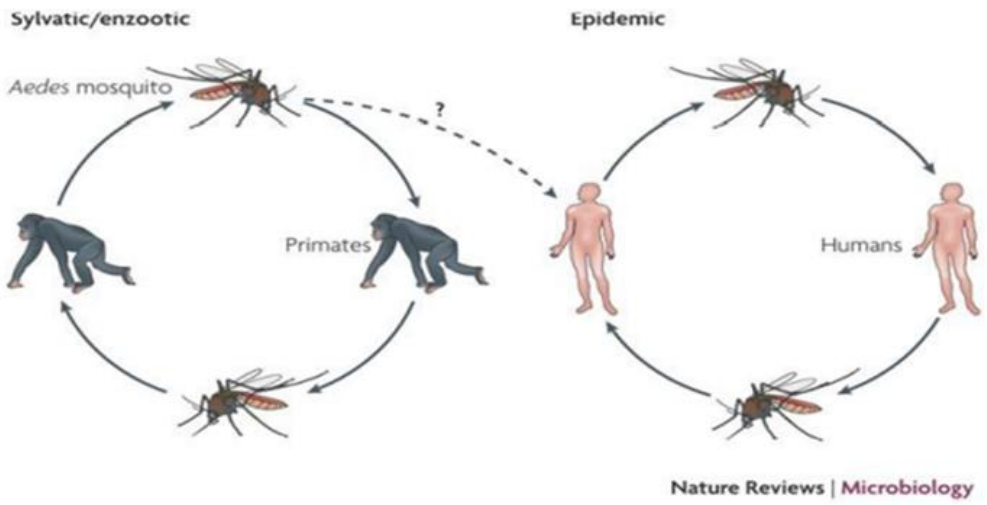


Figure 2.4: Illustration of the Dengue Virus Transmission Cycle

Source: (Chepkorir, 2019)

Both the YFV and DENV ancestral sylvatic cycles are restricted to geographical areas where suitable hosts and vectors are found (Chepkorir, 2019). However, there is a chance of transmission through a viremic traveller, who might come into contact with local competent YFV vectors in a new place, despite the virus's currently limited geographic spread (Hurk *et al.*, 2011).

2.5 Yellow Fever and Dengue Vectors

Some Vectors have been associated with the transmission of YFV through isolation of the virus (Agha *et al.,* 2017b) while other vectors have been assumed due to their population dynamics (blood-feeding preference, climatic conditions and vector abundance), behaviour, and/or capability to transmit YFV under laboratory conditions (Chepkorir, 2019). The YFV epidemics in Central and East Africa are linked with sylvatic mosquito vectors, especially the *Ae. Simpsoni* species complex (*Ae. simpsoni, Ae. Lilii and Ae. Bromeliae*). *Aedes Aegypti* inhabits both sylvatic (non-human-biting) and anthropophilic (human-biting) populations in East Africa, although YFV epidemics have not been documented in regions where *Aedes Aegypti* commonly bite

humans, such as along the Kenyan coast (Chepkorir *et al.,* 2019). Aedes is the main carrier of DENV (Halstead, 2008). Other *Aedes* species mosquitoes that could transmit DENV include *Aedes Africanus, Aedes Albopictus* and *Aedes Luteocephalus*, which are all present in Africa.

The population growth of the *Aedes (Stegomyia)* mosquito species is greatly facilitated by urbanization and the increased human population (Gubler & Clark, 1995). The accumulation of non-biodegradable, man-made containers used to hold water in and around residential areas has created the aquatic habitat to which these mosquitoes are best adapted (Monath, 1994) and waste containers due to poor waste disposal habits (Tigoi *et al.,* 2015). Thus, to ascertain the contributions of each species to DENV and YFV transmission, relevant ecological studies are required in Africa. Both vector surveillance and control, particularly for epidemic transmission control, are essential in the management and prevention of vector-borne diseases. For YFV and DENV, vector surveillance that measures the prevalence of *Aedes aegypti* and other *Aedes Stegomyia species* will assist in determining the likelihood of an urban epidemic (WHO, 2018). A government might prioritize areas for improving its human disease surveillance, laboratory testing for yellow fever, and escalation of vector control measures if it knew the distribution of these mosquitoes within its borders (WHO, 2018).

2.6 Epidemiology of Dengue and Yellow Fever

2.6.1 Burden of Dengue and Yellow Fever Globally

Dengue fever is a well-known viral infection (Chepkorir *et al.,* 2019) and is currently the most significant recurring arboviral illness in the world, with more than 50% of the world's inhabitants at risk for the illness and 50% living in dengue-endemic nations (Inziani *et al.,* 2020; Lutomiah *et al.,* 2016). In over 100 nations in Africa, the Americas, and Asia, 3.6 billion people are currently at risk of contracting dengue (Bhatt *et al.,* 2013; Bonney *et al.,* 2018). About 390 million dengue cases are reported globally, with Africa accounting for 16% of all cases (Bhatt *et al.,* 2013). Furthermore, 0.9 billion individuals are projected to live in YF-endemic areas, with Africa

accounting for nearly 90% of global YFV infections (Ramírez *et al.,* 2018). Currently, Dengue fever is present in over 100 countries across the Western Pacific, Africa, Southeast Asia, the Eastern Mediterranean, and the Americas, putting approximately two-fifths of the world population at risk (Bonney *et al.,* 2018).

2.6.2 Burden of Dengue and Yellow Fever in Africa

According to retrospective serological research carried out by Kokernot, *et al.,* 1956, the initial known dengue outbreak in Africa was in Durban, South Africa, in 1927. In the years that followed, DENV isolations in Africa were documented in the following countries: Sudan (DENV1 and 2) in 1984; Mozambique (DENV3) in 1983-85; Nigeria (DENV1 and 2) in 1964-68; and Senegal (DENV4) in 1986. Since September 2021, human laboratory-confirmed cases of yellow fever have been reported in 9 countries in the WHO African Region, including Ghana, Chad, Cameroon and the Democratic Republic of the Congo. The Republic of Congo, the Central African Republic, Cote d'Ivoire, Niger, and Nigeria are at a higher risk with a history of yellow fever transmission and epidemics (WHO, 2022). In Africa's West and Central regions, these outbreaks are affecting a vast geographic area. These findings show a comeback and escalation in the transmission of the yellow fever virus. The outbreaks have occurred in areas where extensive mass vaccination campaigns have previously been carried out, but where there are ongoing and widening gaps in immunity as a result of human migration (newcomers without a history of vaccination) and/or an absence of continued population immunity through routine vaccination.

2.6.3 Dengue and Yellow Fever in Kenya

Dengue fever virus outbreak (DENV2) was discovered in Kenya's coastal region in Mombasa, Kilifi and Malindi in 1982 (Johnson *et al.,* 1998). This outbreak was believed to come from the outbreak that happened in Seychelles around the years 1979 to 1980 (Konongoi *et al.,* 2016). Yellow fever virus was first detected in Kenya in Kerio Valley Baringo County in 1992-93 (Sanders *et al.,* 1998). Dengue virus and Yellow fever are known to be endemic in Kenya, where they have been detected in low levels to date (Chepkorir *et al.,* 2019; Konongoi *et al.,* 2016; Lutomiah *et al.,*

2013). Seroprevalence investigations carried out in Kenya showed an elevated prevalence of dengue at 34.17 per cent in coastal Malindi and a low incidence at 1.96 per cent in western Busia (Inziani *et al.,* 2020; Mease *et al.,* 2011).

The risk of YFV infections spreading into the country is very high, and YF antibodies have recently been discovered (Chepkorir *et al.,* 2019; Inziani *et al.,* 2020). The most recent epidemic, which occurred in Isiolo county in March 2021, was reported by the Ministry of Health, and as of January 2022, there had been an overall 53 probable yellow fever cases, including six fatalities (case fatality ratio: 11.3%). (WHO, 2022).

2.7 Laboratory Diagnosis of Dengue and Yellow Fever

Arboviruses can be diagnosed using several techniques using a variety of specimens obtained from wildlife, vectors, human samples, and livestock (Chepkorir, 2019; Chepkorir *et al.,* 2014; Tigoi, 2015; Hurk *et al.,* 2011). IgG and IgM antibodies specific to the virus may be detected by serological tests like enzyme-linked immunosorbent assays (ELISA) (Deubel *et al.,* 1983; Johnson *et al.,* 2000; Onyango *et al.,* 2004). Within three to five weeks following the start of an illness, IgM antibody levels reach their peak and remain there for about two months. Cell Culture can also be used to isolate the viruses from blood throughout the first few days of infection (Onyango *et al.,* 2004). Molecular approaches are used to detect the viral nucleic acid in tissue or serum samples obtained during the acute phase of illness (Konongoi *et al.,* 2016; Bonney *et al.,* 2018; Adam and Jassoy, 2021; Mota *et al.,* 2021). RT-PCR results acquired from clinical samples can also be utilized for virus genotyping and serotyping, allowing comparisons between virus samples from different geographical sources (Kuno, 2000). Other techniques for diagnosing arboviruses include the Plaque Reduction Neutralization Test (PRNT), which is considered the gold standard that rules out cross-reactivity within a *Flavivirus* genus, and the Hemagglutination-Inhibition Test utilized in qualitative and quantitative detection of IgG antibodies (Abdullah *et al.,* 2020; Chepkorir *et al.,* 2019; Inziani *et al.,* 2020; Mease *et al.,* 2011; Oladipo et al., 2014). YFV and DENV have been discovered in Kenyan mosquitos (LaBeaud *et al.,* 2011). Antibodies are generally produced in the serum following infection with these viruses (Aniakwaa-bonsu *et al.,* 2021; Inziani *et al.,* 2020; Mease

et al., 2011; Oladipo *et al.,* 2014). Immunoglobulin M (IgM) is produced quickly and is transient, whereas immunoglobulin G (IgG) is produced later and is persistent (Mease *et al.,* 2011). Laboratory abnormalities in severe yellow fever include leukopenia, elevated liver enzymes, hyperbilirubinemia, prolonged coagulation times, and proteinuria. Laboratory findings in severe dengue typically include thrombocytopenia, elevated liver enzymes, and haemoconcentration due to plasma leakage.

2.8 Management of Yellow Fever and Dengue

Management strategies for yellow fever virus and dengue virus involve both clinical care and public health interventions. Clinically, both viruses lack specific antiviral treatments (Silva & Fernandez-Sesma, 2023), and supportive care remains the backbone of the management (Pourzangiabadi *et al.,* 2025; Sinha *et al.,* 2024). For dengue cases, early detection of warning signs, fluid resuscitation, and vigilant monitoring are critical in preventing progression to severe dengue, which can result in plasma leakage, haemorrhage, and shock (World Health Organization, 2023). Similarly, yellow fever management focuses on symptomatic relief, with intensive care required for severe hepatic and renal involvement (World Health Organization, 2025).

Vaccination remains the most effective preventive measure for YFV, with a single dose of the 17D yellow fever vaccine providing long-lasting, often lifelong, immunity and significantly reducing outbreak sizes in endemic countries. However, vaccination coverage remains uneven, especially in low-resource settings (Rodriguez-Morales *et al.,* 2025). Many African countries, including Kenya, face challenges such as limited vaccine supply, logistical difficulties in reaching remote populations, vaccine hesitancy, and weak health infrastructure (Kibathi *et al.,* 2024; Sang *et al.,* 2022). The licensed vaccine for dengue, Dengvaxia® (CYD-TDV), has only been effective in people who have previously been exposed to DENV and has shown poor efficacy in children and dengue-naïve persons, and it has significantly raised the risk of severe dengue in young patients (Silva & Fernandez-Sesma, 2023). The requirement for pre-vaccination screening, the high cost, and worries about safety in recipients who test

negative for the virus have all slowed its rollout, potentially increasing the severity of subsequent infection (Halstead, 2008). When vaccination campaigns are effective, they can significantly lower morbidity and mortality, ease the strain on healthcare systems, and break the cycles of transmission in both urban and sylvatic environments. Nevertheless, intermittent outbreaks have persisted, caused by gaps in vaccine implementation, especially in regions with inadequate surveillance and immunization programs.

Beyond vaccination, integrated vector management plays a pivotal role in reducing the burden of these arboviral diseases. These strategies include community-based mosquito control, environmental sanitation, use of insecticide-treated nets, and novel tools like *Wolbachia*-infected mosquitoes and genetic control approaches. Public education and risk communication have also been instrumental in reducing exposure. However, sustained political commitment and resource allocation are essential to scale these interventions effectively.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Sites

The study was conducted in Kerio Valley and Nguruman both in Baringo and Kajiado Counties respectively as shown in Figure 3.1. The dryland ecosystems of Nguruman and Kerio Valley are comparable, with various livestock and wildlife species, pastoralist groups, and historical evidence of arbovirus circulation (Lwande *et al.*, 2013; Ochieng *et al.,* 2013; Sang *et al.,* 2010). The human population depends on low-lying plains and seasonal rivers for grazing pasture, irrigation water, animal and wildlife sustenance, and a means of subsistence. Both locations include arid African savannah landscapes, dominated by tall grasses, low-lying Acacia shrubs, and irregular trees (Ogola *et al.,* 2023). Nguruman, located in Kajiado County in southern Kenya, borders Tanzania, while Kerio Valley, located in Baringo County, is approximately 250 kilometres northwest of Nairobi.

Kerio Valley in Baringo County was selected because, in 1992/1993, the area recorded the first Yellow fever outbreak in Kenya (Reiter *et al.,* 1998; Sanders *et al.,* 1998) thus there is a possibility of human transmission. Also, the availability of the Yellow fever vectors *Aedes africanus* and Dengue Virus' primary vector *Aedes aegypti* in Kerio Valley makes the area prone to Yellow fever and Dengue fever outbreaks (Lutomiah *et al.,* 2013; Reiter *et al.,* 1998). Nguruman in Kajiado County was selected because there are no studies in the area on YFV and DENV despite having an abundance of vectors for both Dengue virus and Yellow fever (Ogola *et al.,* 2023). In addition, environmental factors that can encourage active transmission in these regions include high temperatures, low rainfall, proximity to lake basins or rivers, flood-prone areas, and forested areas that serve as breeding grounds for vectors as well as habitats for many virus reservoirs.

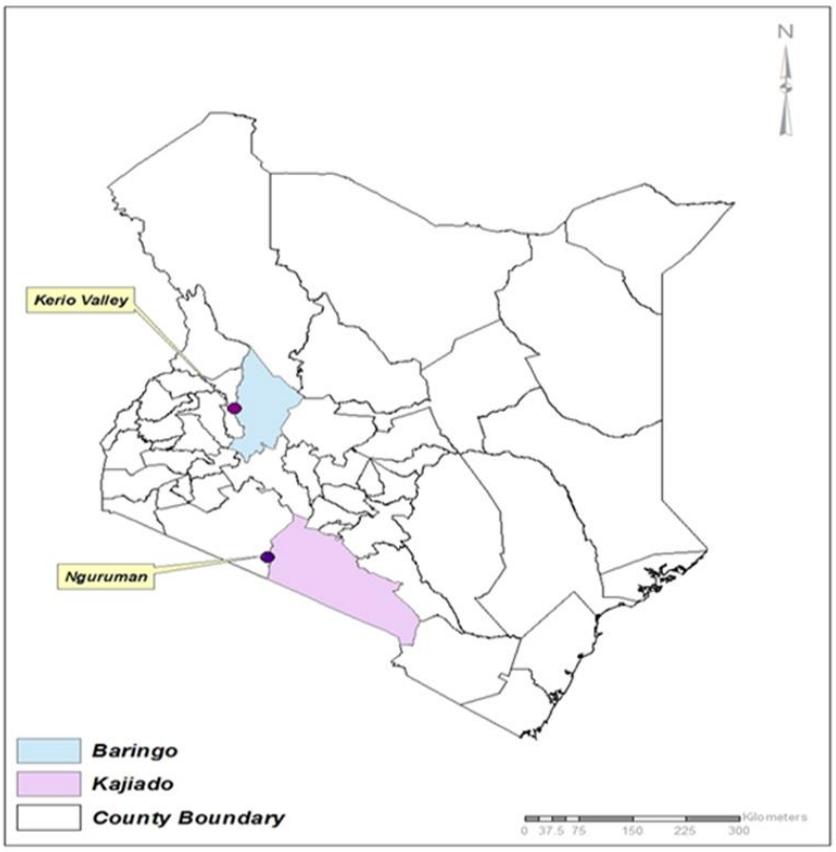


Figure 3.1: The Map of Kenya Showing the Locations of the Two Study Sites: Nguruman in Kajiado South Sub-County and Kerio Valley in Baringo County

3.2 Study Design

The study was a retrospective cross-sectional study nested within an ongoing research project titled "*Epidemiologic Assessment of Risk of Yellow Fever and Dengue Transmission and Outbreaks in Kenya*, Protocol Number: SSC 2787". Thus the samples used were recently collected from Nguruman (Kajiado County) and Kerio Valley (Baringo county) from July 2020 to May 2023.

3.3 Study Populations

This study targeted human populations and *Aedes* mosquitoes of the subgenus *Stegomyia.*

3.4 Sample Size Determination

3.4.1 Human Sample Size Determination

The sample size was calculated using Cochran's (1963) formula for determining sample size in cross-sectional (prevalence) studies (Abdullah *et al.,* 2020).

$$n = \frac{Z^2\,pq}{d^2}$$

Where: n = Minimum sample size, Z = Z statistic, 1.96 for 95% confidence level, P = Estimated prevalence (0.5), q = (1-P) and d= Precision of the estimate required (1-α= 0.05).

$$n = \frac{1.96^2 * 0.5(1-0.5)}{0.05^2} = 384.16$$

$$n = 385$$

Thus, the minimum sample size used was 385. To account for contingencies, the minimum sample size was further increased resulting in a final sample size of 480 individuals.

3.4.2 Mosquito Sample Size

The study's primary goal was to establish the viral circulation to establish the risk level hence to increase the chances of detecting viruses, more mosquito samples need to be screened. For this study, 9183 *Aedes Stegomyia* mosquitoes were collected. The mosquitoes were further grouped into pools containing approximately 5 individual mosquitoes grouped according to species, sex, site and physiological status. Thus, 1822 pools of *Aedes Stegomyia* mosquitoes were tested.

3.5 Human Sample Collection

3.5.1 Participant Recruitment

Participants aged 5 to 85 years presenting with fever (≥38°C) of unknown origin were recruited. A brief clinical history and diagnostic testing were used to exclude malaria and typhoid fever, ensuring that participants had febrile illness of likely viral origin.

3.5.2 Inclusion and Exclusion Criteria for Human Participants

Inclusion Criteria

1) Those participants presenting with febrile illness (≥38°C) not attributable to malaria or typhoid were included in the study
2) Participants who provide informed consent provided in Appendix II and assent for minors shown in Appendix III.
3) Participants who have not been previously vaccinated against yellow fever virus
4) Participants who are aged 5 to 85 years
5) Participants who have resided in the study area for ≥6 months

Exclusion Criteria

1) Participants who refuse to refuse or not willing to provide informed consent or assent form for minors
2) Those participants who have been confirmed positive for malaria or typhoid fever
3) Participants who have prior history of YFV vaccination
4) Participants presenting with severe illness requiring urgent intervention
5) Participants who are below 5 years

3.5.3 Blood Collection and Handling

Approximately 5 ml of venous blood sample was collected aseptically using sterile, single-use vacutainers with clot activator. At the collection site, the blood samples

were allowed to clot at room temperature for 10 minutes and then centrifuged at relative centrifugal force of 112 for 3 minutes to separate serum, which was aliquoted into labelled sterile cryovials. The serum samples were kept at -20^{O}C on site and transported in a liquid nitrogen shipper to the Kenya Medical research institute (KEMRI) laboratory. Upon arrival at KEMRI, the serum samples were stored at -80^{O}C awaiting serological analysis. Demographic information about participants' age, gender, occupation, status of yellow fever vaccination, place of residence, and any pertinent travel history were collected using demographic data sheet as shown in appendix III.

3.6 Adult Mosquito and Larvae Trapping and Collection Procedure

Mosquitoes were collected from peridomestic areas of Kerio Valley and Nguruman. These areas included tree holes, abandoned tins, discarded tyres, discarded food tins, broken basin with bottles, and drum tops. The adult mosquitoes and larvae were collected shortly after the short and long rains when there is high mosquito activity is anticipated (Agha *et al.,* 2017a). At each site, ten traps of each type (BG-Sentinel during the day between 6 am and 6 pm and CDC light traps during the night set at 6pm and retrieved at 6 am) were used daily, positioned in peridomestic areas. CO_2 was supplied in the form of dry ice, approximately 2 kg per trap per day, dispensed in 2-liter Thermos flasks with small outlet holes to allow for gradual sublimation throughout the trapping period.

In addition, immature stages (larvae and pupae) of mosquitoes were collected from the sites using standard larval sampling tools, including ladles, droppers/pipettes, and ovicups. Collected larvae, pupae were reared to adulthood under controlled environmental conditions in field laboratories. The reared adult mosquitoes, particularly *Aedes (Stegomyia)* species, were anesthetized using triethylamine (TEA) vapour in the field, a safe and effective knockdown agent for preserving mosquito integrity. Mosquitoes were immediately stored in liquid nitrogen in the field and then transferred to −80 °C freezers upon arrival at the KEMRI Laboratory.

The species of mosquitoes were identified under a dissecting microscope based on the known taxonomic keys (Coetzee, 2020; Gillies & Coetzee, 1987; Gyawali *et al.*, 2025; Hington, 1983; Huang, 2004). Adults were identified by their dark bodies with white or silver scales on the legs and thorax, often forming a lyre-shaped pattern on the thorax. The identified mosquitoes were pooled based on sex, species and site of collection in pools containing approximately 5 individual mosquitoes per pool. Each mosquito pool was placed into a sterile 2 mL screw-cap and stored in -80^{O}C freezer awaiting further processing

3.7 Laboratory Procedures

3.7.1 Yellow Fever Virus and Dengue Virus Amplification

The neutralization was performed utilizing live preserved viral isolates of DENV-2 (008/01/2012) passage 2 and YFV passage 3 (YFXSMB) sourced from KEMRI's Viral Hemorrhagic Laboratory. The viruses were propagated in T-75 cm^2 flasks with a confluent monolayer of C6/36 cell lines (passage 2) derived from *Aedes albopictus* mosquitoes grown in Dulbecco's Modified Eagle Medium (DMEM) (GIBCO® Invitrogen Corporation, Carlsbad, CA, USA) containing 4.5 g/L D-glucose, without L-glutamine. The medium was supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Sigma-Aldrich, St. Louis, MO, USA), 2% L-glutamine (Sigma-Aldrich), and 2% antibiotic/antimycotic solution (Sigma-Aldrich) containing 10,000 units/ml penicillin, 10 mg/ml streptomycin, and 25 µg/ml amphotericin B. Cells were incubated overnight at 28 °C in a humidified atmosphere with 5% CO_2 prior to virus infection.

Briefly, 600µl of the virus was added the confluent monolayer of C636 cells in T-75 cm^2 flask. The flask was incubated for one hour with rocking after every 15 minutes to enable virus adsorption. The cells were then maintained in Dulbecco's modified Eagles medium (DMEM) and observed daily to check for cytopathic effects (CPE). When 80% of the cells' monolayer showed CPE, the flasks were removed from the incubator and frozen overnight at - 80^{O}C. the flasks were retrieved, thawed on ice and centrifuged at 1500 revolutions per minute for 10 minutes to obtain a clear supernatant.

The supernatant was harvested and aliquoted into 1.5 ml cryovials and stored at -80^{O}C awaiting quantification by plaque assay (Baer & Kehn-Hall, 2014).

3.7.2 Yellow Fever Virus and Dengue Virus Quantification Using Plaque Assay

Plaque assay was used to quantify the viruses harvested, following the method described by Baer & Kehn-Hall (2014). To begin, serial dilutions of the viral stocks were performed in ten-fold dilutions. Microcentrifuge tubes (2.0 ml capacity, Eppendorf, USA) were labelled with concentrations ranging from 10^{-1} to 10^{-6}, along with a Negative control. Each tube received 900 µl of maintenance media, which was a mixture of Minimum Essential Media (Sigma) enhanced with L glutamine (2%), heat-inactivated fetal bovine serum (FBS) (2%), and penicillin/amphotericin B ((2%). Serial dilutions were done by pipetting 100 microlitres of the virus into the 10^{-1} tube and vortexing it. From there, 100 µl was transferred to the next tube, continuing until the 10^{-6} dilution, after which the remaining 100 µl was discarded.

E6 Vero cells (passage 3) were carefully seeded onto a 12-well plate and cultured for 24 hours in a 5% CO2 incubator at 37°C to ensure a confluent monolayer was formed. The wells of the seeded 12–well plate were labelled as; Negative control, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, and 10^{-1}. 100 microlitres of the diluted virus was then added to the respective labelled wells, making sure to change the pipette tip after each use. The plate was then incubated for an hour at 37°C in 5% CO2, with gentle agitation every half an hour to help the virus bind to the cells.

After this adsorption period, a 1ml overlay of 2.5% methylcellulose was added to the wells and incubated in a 5% CO2 environment at 37°C. Between 6 – 14 days, the plates were fixed using formalin and subsequently stained with diluted crystal violet to make the plaques visible. Finally, the formed plaques were counted and quantified using the below formula (Baer & Kehn-Hall, 2014; Gargan *et al.*, 1983).

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

where d represents the dilution factor and V denotes diluted virus volume.

The titre of the virus was measured in terms of plaque-forming units (pfu) per ml, utilizing a suitable virus-dilution factor that yielded between 20 and 70 plaques (Baer & Kehn-Hall, 2014) to determine the necessary amount of virus for dilution intended for PRNT. Table 3.1 displays the determined viral titers for the viruses used in this study.

Table 3.1: Dengue -2 and Yellow Fever Viral Titres Determined by Plaque Assay

Viruses	Number of Passage	No. of Plaques	Dilution Factor	Amount of Diluted Virus Added (µl)	Titre (pfu/ml)
YFV(YFXSMB)	2	82	10^{-3}	100	$8.2X10^{5}$
DENV-2 (008/01/2012)	1	48	10^{-2}	100	$4.8X10^{4}$

DENV-2, Dengue-2 virus; YFV, Yellow fever virus; pfu, plaque forming units

3.7.3 Plaque Reduction Neutralization Test (PRNT)

PRNT used Vero E6 cell lines (passage 3) for viral culture. All the serum samples collected were heat-inactivated at 56°C for 30 minutes. 24 well culture plates were labelled. Vero cells (E6 or CCL-81 cell lines) were seeded into the 24 well culture plates. To evenly distribute cells on the plate surface, the seeded plate was rocked back and forth, then side to side. The plate was incubated overnight to allow cells ($1x10^{6}$ cells/well) to adhere. The plates were examined under a light microscope after overnight incubation to confirm 70-90 per cent confluence and even cell distribution. Serum samples were aliquoted into 30 µl vials and diluted at 1:10 using maintenance media (MEM) to determine the endpoint titre, or the highest dilution capable of neutralizing at least 99 per cent of the virus at 1:10 to 1:5120. In the first well, 6µl of a serum sample was added to the 54µl of maintenance media and mixed thoroughly. The MEME was enhanced with fetal bovine serum (2%), L-glutamine (2%) and Antibiotic/antimycotic (2%). The second well was filled with 30µl of the 1/10 serum dilution (1:20 dilution). This process was repeated for subsequent dilutions, with the last 30µl discarded at the end of the titration. Following the Plaque Assay results, maintenance media was used to dilute the virus to ensure 20-70 plaque-forming units

per ml 30µl of the diluted virus was dispensed in to each well. The plates were covered and gently mixed before being incubated for 1 hour at 37 degrees' Celsius incubator. After that, the antibody-virus mixture was inoculated onto plate with a confluent E6 cells and placed in an incubator (37 degrees Celsius in a 5% CO_2) for 1 hour to allow virus adsorption. Following the adsorption process, the wells were covered with a methylcellulose (2.5%) (Sigma) overlay mixed with 2X Minimum Essential Medium (Sigma). The plaques were fixed with formalin, stained with crystal violet diluted in absolute ethanol, and manually counted within 6-14 days after inoculation (Odhiambo *et al.,* 2015; Tigoi *et al.,* 2015). The end-point titers of the plaque reduction neutralisation test (PRNT), which indicate seropositivity thresholds, are represented as the reciprocal of the final serum dilution that demonstrates the required percentage reduction in plaque counts (WHO, 2007). In this investigation, the endpoint titre was documented as the reciprocal of the maximum serum dilution that achieved a ≥ 90 % reduction in plaque counts (PRNT90) (Roehrig *et al.,* 2008). In this study, PRNT90 was employed to determine the maximum serum dilution (ranging from 1:10 to 1:5160) necessary to achieve a 90% reduction in plaque formation in Vero cells (Ribeiro *et al.,* 2020). When several viruses neutralized the same serum sample, the virus with an antibody titer four times or higher than the other flaviviruses tested was regarded as virus-specific and positive, in accordance with the criteria and guidelines set by the WHO (WHO, 2007).

3.7.4 Testing Virus Activity in Mosquitoes

3.7.4.1 Homogenizing the Mosquitoes

Pooled mosquitoes (approximately 5 mosquitoes per pool) were retrieved from the freezer. A 4.5mm copper-shot beaded ball was added to the microcentrifuge tube. 1 ml of homogenizing media was added to the tube. The microcentrifuge tube was placed in a Mini-Beadruptor-16 (Biospec, Bartlesville, OK, USA) at a speed of 2.10 for 20 seconds to crash the mosquitoes. The ground mosquito homogenate was centrifuged at 4°C and 12000 revolutions per minute for 10 minutes. The resulting supernatant was aliquoted into sterile cryovials and immediately stored at −80 °C pending subsequent analysis.

3.7.4.2 Virus Isolation (Inoculation onto Cells)

24 well plates containing Vero cell monolayers at 75% - 85% confluence, were inoculated with 50 µl of the harvested supernatant. A negative control (50 µl of maintenance media) was set in one of the wells. 50 µl of a previously isolated YFV (YFXSMB) was also included as a positive control. The plates were incubated for one hour at 37 degrees Celsius for adsorption, then overlaid with 1ml of maintenance media (MEM) and incubated in a 5% CO2 incubator at 37°C. The plates were monitored daily for 14 days for cytopathic effects (CPE). Wells showing CPE were harvested and the content was transferred to a cryogenic vial and frozen at -65°C to -85°C awaiting passaging to confirm the cytopathic effect. A viral isolate was suspected when a passage of the originally harvested culture showed reproducible cytopathic effect. The suspected isolates were processed for viral RNA extraction, reverse transcription-polymerase chain reaction (RT-PCR), gel electrophoresis, and Sanger sequencing.

3.7.4.3 RNA Extraction and Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Viral particles obtained from cultures that tested positive for CPE were filtered using 0.22µm filters (Millipore, Merck) to eliminate larger particles such as bacteria and concentrate the virus particles. Viral RNA was extracted directly from 140µl filtrate using the QIAamp Viral RNA Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. Briefly, 140 µL of the sample was added to 560 µL of Buffer AVL containing carrier RNA in a microcentrifuge tube. The mixture was pulse-vortexed vigorously for 15 seconds to ensure efficient lysis and incubated at room temperature for 10 minutes. The tube was briefly centrifuged to remove drops from the inside of the lid. An equal volume (560 µL) of molecular grade ethanol (96–100%) was then added to the lysate and mixed thoroughly by pulse-vortexing for 15 s. the tubes were again, briefly centrifuged briefly to remove drops from inside the lid. 630 µl of the solution was carefully added to the QIAamp Mini column (in a 2 ml collection tube) without wetting the rim. The cap was closed and centrifuged at 8000 rpm for 1 min at room temperature. The QIAamp Mini column was placed into a clean 2 ml

collection tube and the flow-through was discarded. 500 µl of buffer AW1 was added to the column and centrifuged at 8000 rpm for 1 min. The column was placed in a clean 2 ml collection tube and the tube containing the filtrate discarded. 500µl of buffer AW2 was then added to the column and centrifuged at full speed (14,000 rpm) for 3 min followed by second was with buffer AW2. The column was then placed in a clean 2 ml microcentrifuge tube, and the RNA was eluted by adding 60 µl Buffer AVE directly to the membrane of the column. The column was incubated at room temperature for 1 minute followed by centrifugation at 8000 rpm for 1 minute. The 60µl of the eluted viral RNA was for used for subsequent complementary DNA (cDNA) synthesis. Firstly, a Reverse transcription reaction was used to synthesize the first strand of complementary DNA (cDNA) from extracted viral RNA using Applied Biosystems cDNA reverse transcription kit (Thermo Fisher Scientific) with random primers. This yielded 20µl of cDNA. The cDNA was amplified by PCR using 2 universal primers for the genus *Flavivirus;* FU_1 5'- TAC AAC ATG ATG GGA AAG AGA GAG AA-3' and CFD_2 5'- GTG TCC CAG CCG GCG GTG TCA TCA GC-3' *(*The main target being DENV, YFV, Zika and WNV) (Konongoi *et al.,* 2018), Two universal primers for *Alphavirus family;* VIR2052F 5'-TGG CGC TAT GAT GAA ATC TGG AAT GTT-3' and VIR2052R 5'-TAC GAT GTT GTC GTC GCC GAT GAA-3') (The main targets are O'nyong nyong virus (ONNV), CHIKV) (Konongoi *et al.,* 2018), *Phlebovirus* (Target is RVFV) and *Orthobunyavirus* (Iwashita *et al.,* 2018; Ochieng *et al.,* 2013; Onyango *et al.,* 2004) (Table 3.2). The PCR reactions were carried out on a Perkin Elmer GeneAmp 9700 Thermocycler (Applied Biosystems, Warrington, England). An appropriate positive control and a negative control cDNA were added to each PCR experiment.

Table 3.2: Primer Sequences, Their Target Genes and Positions

Virus Family	Primer	Primer Sequence	Cycling Conditions	Reference
Flavivirus	FU1	5′-TACAACATG ATGGGAAAGA GAGAG AA-3′)	95°C(10min) 95°C (30sec) 55°C(30sec)	(Konongoi *et al.,* 2018)
	CFD2	5′-GTGTCCCAG CCGGCGGTG TCATCA GC-3’	68°C (45sec) for 35 cycles 72°C (7 min) 4°C (hold)	
Alphavirus	Vir2052F	5′-TGGCGCTAT GATGAAATCT GGAAT GTT-3’	95°C (10min) 95°C (30 sec) 49°C (30sec)	(Konongoi *et al.,* 2018)
	Vir2052R	5′-TACGATGT TGTCGTCGCC GATGAA3′	72°C (30sec) for 35 cycles 72°C (10 min) 4°C (hold)	
Orthobunyavirus	BCS82C	5′ ATGACTGAGT TGGAGTTTCATG ATGTCGC 3′	95°C (10 min) 95°C (30sec)	(Eastwood *et al.,* 2020)
	BCS332V	5′ TGTTCCTGTT GCCAG GAAAAT3′	55°C (30sec) 68°C (45sec) for 35 cycles 72°C (7 min) 4°C (hold)	

3.7.4.4 Visualization Using Gel Electrophoresis

The amplified PCR product was visualized using agarose gel electrophoresis (Khan *et al.,* 2020). A 2% agarose gel was prepared by dissolving 2 grams of agarose powder (Sigma-Aldrich, St. Louis, MO, USA) in 100 ml of 1×Tris-acetate-EDTA (TAE) buffer. The mixture was heated in a microwave oven for 3 minutes until the agarose was completely dissolved, then allowed to cool to approximately 60°C. About 7 µL of nucleic acid stain (ethidium bromide) was added to the gel solution to enable visualization of DNA under UV light. The molten gel was poured into a gel casting tray with an inserted comb and allowed to solidify at room temperature for 20–30 minutes. After the gel had solidified, it was placed into an electrophoresis tank and completely submerged in 1×TAE buffer. PCR products were mixed with 6×DNA loading dye. A 5 µL aliquot of each PCR product was loaded into individual wells of the gel alongside a 1 kb DNA ladder (Thermo Fisher Scientific, USA) to estimate fragment sizes. Electrophoresis was carried out at a constant voltage of 120 volts for

approximately 30 minutes, or until the dye front had migrated about three-quarters of the gel length. After electrophoresis, the gel was transferred to a UV transilluminator for visualization of DNA bands. Amplification was considered successful when a discrete band corresponding to the expected amplicon size was observed in the test lanes. Images of the gels were captured and archived for documentation and analysis. The samples that showed clear bands were processed for sequencing.

3.7.4.5 DNA Sequencing

The amplified target DNA bands were purified directly from the PCR reaction using ExoSAP-IT (Thermo Fisher Scientific), a two-enzyme system composed of Exonuclease I and Shrimp Alkaline Phosphatase (SAP) following manufacturer-recommended protocols. Briefly, 5 µl of PCR product and 2 µl of ExoSAP-IT were mixed, incubated for 15 minutes at 37°C to degrade any residual PCR primers and dephosphorylate excess dNTPs after amplification. This step was followed by enzyme inactivation at 80°C for 15 minutes.

The purified PCR products were used as templates for Sanger sequencing reactions. A 10µL reaction comprising BigDye Terminator v3.1 (Applied Biosystems), 5x sequencing buffer, 3.2 pmol of either forward or reverse primer, and purified PCR product was used for sequencing. The thermal cycling conditions were as follows: initial denaturation at 96°C for 1 minute, followed by 25 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 4 minutes. After sequencing, products were purified using ethanol/EDTA precipitation to remove unincorporated dyes followed by capillary electrophoresis using ABI 3500 Genetic Analyzer (Applied Biosystems). Chromas Version 2.6.6 was used to trim low-quality end reads whereby the generated chromatogram files were edited using BioEdit version 7.2.5 to generate consensus sequences (Hall, 1999).

3.8 Data Management

All the data collected in this study was labelled with only specific ID numbers (also known as code numbers). Thus, all patients' information remained confidential. Additionally, no participants' personal information i.e., names was used in any report

of this study, publications or presentations. The soft copy data was stored in password-protected folders in a computer designated for this study. Antivirus was installed in the computer to avoid destruction by viruses. Hard copy data was kept in lockable cabinets. Backup of the data was done and kept in a password-protected external hard disk and flash drive. The samples were stored in the -80^{O}C freezer.

3.9 Data Analysis

The analysis of data was done using version 4.3.2 of the R statistical software (R Core Team, 2020) and Microsoft Excel. Human data was analyzed and seroprevalence was compared. Using age, gender, and occupation as covariates, a multinomial logistic regression model was used to forecast risk for each of the most common viruses. The correlation between the outcomes and the risk factors was evaluated using the odds ratio (OR). For mosquito data, analysis was done by compiling the collections by site and collection method (BG sentinel and CDC-light traps and traps). An analysis of the abundance of mosquito species was also conducted. The chi-square test was used to evaluate and compare the proportions among the sites for both human and mosquito data. A p-value of ≤0.05 was regarded as statistically significant at a 95% confidence interval.

Sequences acquired were analyzed the Basic Local Alignment Search Tool (BLAST) from NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic analysis was conducted using Molecular Evolutionary Genetics Analysis (MEGA) software version 7.0 (Kumar *et al.*, 2016). Different sequences belonging to the virus families tested were downloaded from GenBank and combined with sequences obtained from this study to conduct analysis. Alignment was conducted using Muscle v6 embedded in MEGA11 after which the Maximum Likelihood approach in MEGA11 was used for phylogenetic analysis and construction of phylogenetic trees (Tamura *et al.,* 2021).

3.6 Ethical Consideration

The study received approval from the Kenya Medical Research Institute's (KEMRI) Scientific Ethics and Review Unit (SERU) under protocol number

KEMRI/SERU/CVR/013/4664 as shown in Appendix IV, as well as from the National Commission for Science, Technology & Innovation, with Research License number NACOSTI/P/23/25147 (see appendix V). Additionally, permission to use the samples was obtained from the principal investigator as shown in Appendix VI.

CHAPTER FOUR

RESULTS

4.1 Study Participant's Demographic Characteristics

The participant's demographic data is shown in Table 4.1. There were 480 participants in this study; Nguruman had 40.4% (194/480) while Kerio Valley had 59.6% (286/480). Women comprised 60.21% (289/480) and men constituted 39.79% (191/480). The participants were between the ages of 5 and 85, with a mean age of 27. Farmers comprised 37.16% of the participants, followed by students (30.48%) and businesses (5.64). 16.08% of the population was employed as housewives, 8.98% of the participants had no listed occupation while 1.67% engaged in other economic activities. Most participants (56.25%) were below 25 years of age, with the majority being students. All the 480 participants were not vaccinated against yellow fever virus.

Table 4.1: Demographic Characteristics of Study Participants from Kerio Valley and Nguruman

Characteristic	Nguruman		Kerio Valley		Combined	
	n	%	n	%	n	%
All	194	100	286	100	480	100
Sex						
Male	99	51.03	92	32.17	191	39.79
Female	95	48.97	194	67.83	289	60.21
Age Group (Years)						
Below 25	102	52.6	168	58.74	270	56.25
25-45	75	38.7	69	24.13	144	30
46-66	16	8.2	37	12.94	53	11.04
66 and above	1	0.5	12	4.2	13	2.71
YF Vaccination Status						
Non- vaccinated	194	100	286	100	480	100
Occupation						
Farmer	100	51.55	78	27.27	178	37.16
Student	38	19.59	108	37.76	146	30.48
Businessman	4	2.06	11	3.85	15	3.13
Business lady	4	2.06	9	3.15	12	2.51
Housewife	16	8.25	61	21.33	77	16.08
Others	5	2.58	3	1.05	8	1.67
Non	27	13.92	16	5.59	43	8.98

4.2 Dengue and Yellow Fever Neutralizing Antibody Seroprevalence in Nguruman and Kerio Valley

Neutralizing antibodies to at least one of the *Flaviviruses* examined were found in 33.13%; 159/480 (95% CI, 50.1–59.0%) of the total samples, with larger proportions found in Kerio Valley (30.2%, 145/480) than in Nguruman (2.92%, 14/480) (P<0.0001). Generally, seropositivity was highest for yellow fever virus (32.5%) compared to DENV-2 (0.63%) (Table 4.2). The prevalence of dengue virus and yellow fever virus neutralizing antibodies varied by study location. YFV seropositivity was significantly greater in Kerio Valley (51%) compared to Nguruman with 6% (P<0.0001). DENV-2 neutralizing antibodies were only detected in Nguruman with a low percentage of 1.5% (Table 4.2).

Table 4.2: Dengue-2 and Yellow Fever Neutralizing Antibody Seroprevalence

Virus	Kerio Valley n(%)	Nguruman n(%)	Combined n(%)
YFV	145 (51%)*	11 (6%)	156 (32.5%)
DENV-2	0 (0%)	3 (2%)	3 (0.63%)
Total +ve	145 (30.2%)	14 (2.92%)	159 (33.13%)
Negative	141(29.38%)	180 (37.5)	321(66.88%)

YFV, Yellow fever virus, DENV-2, Dengue-2 virus
* Chi-square test's significant difference (p<0.0001) between the sites

Based on gender, figure 4.1 The percentage of samples with dengue and yellow fever-neutralizing antibodies by gender in Kerio Valley and Nguruman with error bars representing the 95% confidence interval. The results show that in Kerio Valley, females' seropositivity (92/286; 32.16%) was substantially greater than males' (53/286; 18.53%) (P<0001). However, the seroprevalence was higher in males (11/194; 24.23%) from Nguruman as compared to females (1.55%; 3/194) P=0.049).

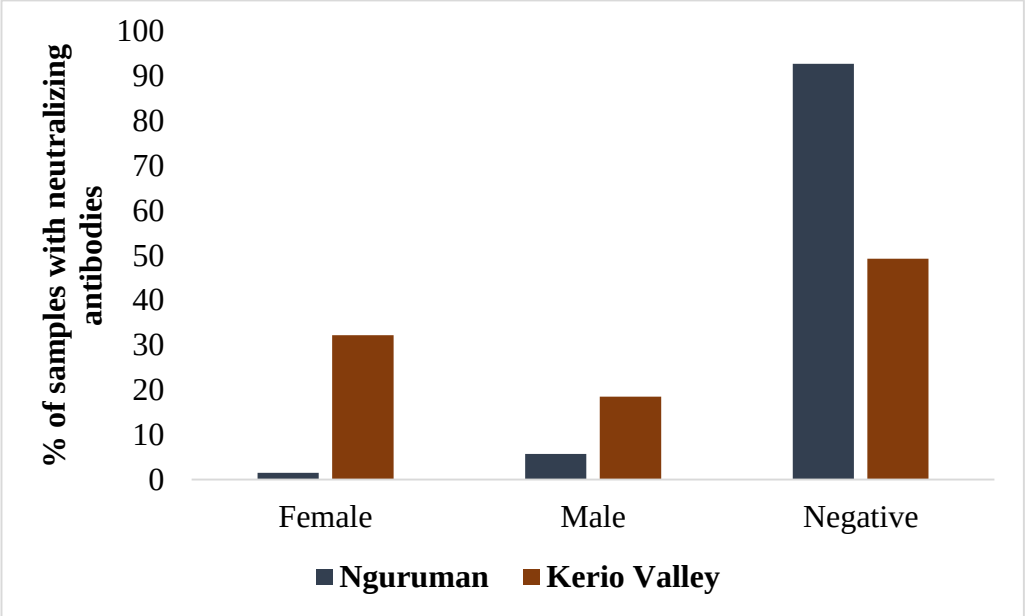


Figure 4.1: The Percentage of Samples with Dengue and Yellow Fever-Neutralizing Antibodies by Gender in Kerio Valley and Nguruman

The mean age of participants with YFV-neutralizing antibodies in Kerio Valley was 28.7 while the mean age of participants without YFV-neutralizing antibodies was 25.98. In Nguruman, the mean age of participants with YFV-neutralizing antibodies was 27.2, while those with DENV-neutralizing antibodies had a mean age of 30.7. The participants without neutralizing antibodies to either YFV or DENV had a mean age of 26.4 (Figure 4.2).

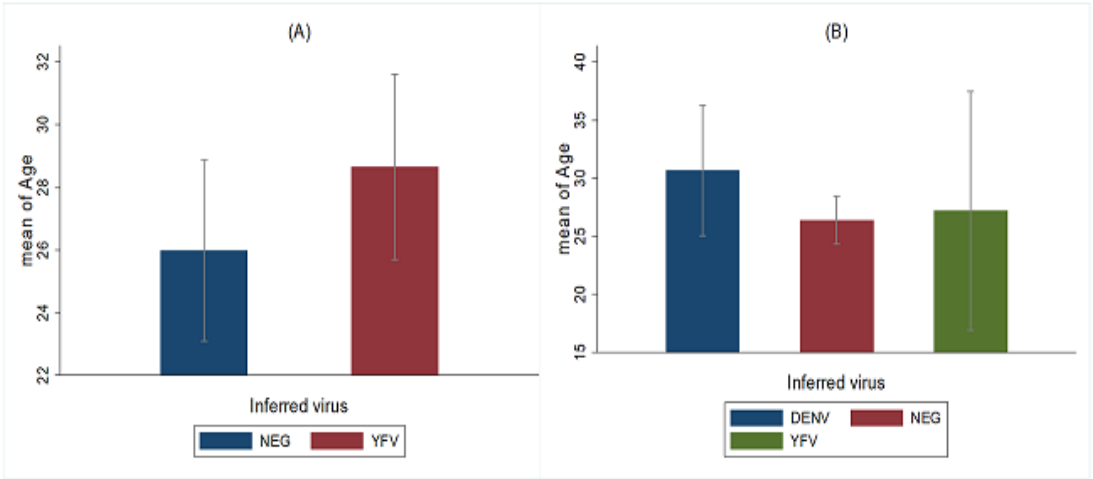


Figure 4.2: Comparison of Means of Age With the Inferred Flaviviruses in Kerio Valley (A) and Nguruman (B) With Error Bars Representing the 95% Confidence Interval

Age group differences were also observed in the neutralizing antibodies against the two viruses (Table 4.3). In Kerio Valley, the prevalence of YFV-neutralizing antibodies was significantly correlated with age (P value = 0.043). Those participants in the age group 25years and below recorded the highest percentage of YFV neutralizing antibodies (25.9%, 74/286) followed by the 26-45 years' age group (15.4%, 44/286), 46-65 years' age group with (7%, 20/286) and finally the 66+ years age group (2.4%, 7/286). A similar trend was also seen in Nguruman whereby yellow fever neutralizing antibodies prevalence was high among the participants of age 25 years and below (3.1%,6/194) followed by the 26-45 years' age group (1.5%, 3/194) and lowest in the 46-65 years' age group (1%,2/194). There were no YFV- neutralizing antibodies observed in the 66 and above age group. Neutralizing antibodies to DENV were only observed in Nguruman whereby the prevalence of DENV-2 neutralizing antibodies was highest in the age group 26–45 years and lowest in the below 25 years' age group. There were no DENV- neutralizing antibodies observed in either 46-65 and 66 and above age groups as shown in Table 4.3

Table 4.3: Proportion of Samples With Yellow Fever and Dengue Virus Neutralizing Antibodies Categorized by Age Groups in Kerio Valley (N=286) and Nguruman (N =194)

Age groups (in years)	Nguruman		Kerio Valley	
	YFV n (%)	DENV n (%)	YFV n (%)	DENV n(%)
Below 25 Yrs	6 (3.1)	1 (0.52)	74 (25.9)	0 (0)
26-45 yrs	3 (1.5)	2 (1)	44 (15.4)	0 (0)
46-65 yrs	2 (1)	0 (0)	20 (7)	0 (0)
66+ yrs	0 (0)	0 (0)	7 (2.4)	0 (0)
Total	11 (6)	3 (2)	145 (51)	0 (0)

DENV-2 (Dengue-2 virus); YFV (Yellow fever virus)

4.3 Demographic Determinants for Prevalence of YFV and DENV Neutralizing Antibodies

Results obtained from the Multinomial Logistic Regression model are displayed in Table 4.4. According to the data, males in Kerio Valley had a roughly two-fold higher likelihood than females of having contact with the yellow fever virus (OR =1.68, 95%, CI = 0.97-2.91, P value = 0.04). The results further suggested that the participants in the 26–45 years' age group were 2.67 times more likely to have YFV- neutralizing antibodies than those who are 25 years and below (OR = 2.67, CI = 1.25-5.69, P value = 0.01). The results show that age and sex were significantly associated with YFV-neutralizing antibodies whereas occupation had no significant association with YFV-neutralizing antibodies' prevalence in Kerio Valley. In Nguruman, there was about a two times increase in the chance of DENV- neutralizing antibodies in males compared to females (OR=2.19, CI = 0.19 -24.75) however, the relationship was not statistically significant (P-value = 0.79). Farmers were around 1.42 times greater chance of having YFV-neutralizing antibodies (OR = 1.42, CI = 0.02-9.33) than Businessmen/women while students had a 1.56 times more chance of having YFV-neutralizing antibodies.

Table 4.4: A Comparison of YFV and DENV-2 Predictors by Age, Occupation, and Gender Using a Multinomial Logistic Regression Model

Variable	Kerio Valley Yellow fever		Nguruman Yellow fever		Dengue	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Sex						
Female	Reference		Reference		Reference	
Male	1.68 (0.97-2.91)	0.043*	0.61 (0.13 - 26.75)	0.79	2.19 (0.19 - 24.75)	0.525
Age						
25yrs & below	Reference		Reference		Reference	
26-45yrs	2.67 (1.25-5.69)	0.011*	0.81 (0.27-2.43)	0.7	0.46 (0.29 - 7.29)	0.586
46-65yrs	1.76 (0.72 - 4.31)	0.22	0.30 (0.03-2.73)	0.3	0.28 (0.41-12.8)	0.99
66yrs & above	1.86 (0.50 - 6.96	0.35	0.00 (0.00-0.00)	>0.9	0.00 (0.00-0.00)	>0.9
Occupation						
Business	Reference		Reference			
Farmer	0.37 (0.27 - 2.02)	0.554	1.42 (0.02-9.33)	0.6	0.24 (0.04-1.62)	0.14
Housewife	0.83 (0.28 - 2.5)	0.746	0.51 (0.05-5.25)	0.6	0.45 (0.10-1.94)	0.3
Student	0.94 (0.36 - 2.44)	0.904	1.56 (0.15-16.3)	0.7	0.69 (0.15-3.15)	0.6

*Indicates a statistical significance at a 95% confidence level

4.4 Determination of Viral Activity in *Aedes Stegomyia* Mosquitoes Collected from Kerio Valley and Nguruman, Kenya

4.4.1 Mosquito and Larvae Abundance

7739 adult mosquitoes were collected using BG sentinel and CDC-light traps from both sites; 5317 (68.7 %) from Nguruman and 2422 (31.3%) from Kerio Valley. 1444 larvae were also collected with 1186 (82.13%) from Nguruman and 258 (17.87%) from Kerio Valley. More mosquitoes were collected in Nguruman (n=5317, 68.7%) than in Kerio Valley (n=2422, 31.3%). Most of the mosquitoes were captured in BG sentinel traps (6280, 81.1%), followed by CDC light traps (n = 1459, 18.9%). (Table 4.5). The adult mosquitoes and larvae were further divided into pools based on sex, site and collection method. A total of 1691 pools of adult mosquitoes were obtained; 1013 (59.9%) from Nguruman and 678 (40.1%) from Kerio Valley. Further, 131 pools of

larvae were obtained with 89 (67.9%) from Nguruman and 42 (32.1%) from Kerio Valley (Table 4.5)

Table 4.5: Adult Mosquito and Larvae Abundance by Sampling Method (BG-Sentinel and CDC Light Traps)

Collection Method	Site		
	Nguruman N (%)	**Kerio Valley N (%)**	**Combined N (%)**
Total Adult Mosquitoes			
BG sentinel traps	4131 (77.7)	2149 (88.7)	6280 (81.1)
CDC-light traps	1186 (22.3)	273 (11.3)	1459 (18.9)
Total	5317 (68.7)	2422 (31.3)	7739 (100
Pools of Adult Mosquito			
BG sentinel traps	566 (55.9)	522 (77)	1088 (64.3)
CDC-light traps	447 (44.1)	156 (23)	603 (35.7)
Total	1013 (59.9)	678 (40.1)	1691(100)
Larvae			
Total larvae collected	1186 (82.13%)	258 (17.87%)	1444 (100)
Total larvae pools	89 (67.9%)	42 (32.1%)	131 (100)

4.4.2 *Aedes stegomyia* Species Diversity and Distribution

4.4.2.1 The Diversity and Distribution of Adult Mosquito Species

The mosquitoes collected were taxonomically classified into 10 species within the genus *Stegomyia* (Table 4.6). *Aedes aegypti, Aedes simpsoni, Aedes metallicus and Aedes africanus* were the most dominant species among the mosquito fauna sampled. Overall, *Aedes aegypti* (49.85%) was the highest followed by *Aedes metallicus* (16.5%), *Aedes simpsoni* (14.78%), *Aedes stegomyia spp.* (7.94%), *Aedes chausseri* (2.37%), *Aedes africanus* (1.7%), with *Ae. Hirsutus*, *Ae. Tarsalis*, *Ae. Furcifer, Ae. Aedimorphus spp.* Recording less than 1 per cent each (Table 4.6). Nguruman reported the greatest number of mosquito species (10 species) while Kerio Valley had only six species. The most dominant species in Nguruman and Kerio Valley was *Aedes aegypti,* 52.9% (536/1013) and 45.3% (307/89) respectively.

4.4.2.2 Larval Species Diversity and Distribution

The 131 pools of mosquito larvae belonged to five species with the majority being *Ae. Aegypti* and *Ae. Simpsoni* (Table 4.6). Overall, Aedes aegypti (68.7%) was the highest followed by *Aedes simpsoni* (16.8%), *Aedes metallicus* (16.1%), *Aedes stegomyia spp.* (3.8 %), and lastly *Aedes africanus* and *Aedes chausseri* both at 2.3%. In Nguruman, only 3 species were identified with the most predominant being *Aedes aegypti* (76.4%) followed by *Aedes simpsoni* (12.4%), *Aedes metallicus* (9%). *Aedes metallicus* was only recorded in Nguruman. On the other hand, Kerio Valley recorded all five species with *Aedes aegypti* recording the largest percentage (52.4%) among the five species followed by *Aedes simpsoni* (26.2%), *Aedes africanus* (7.1%), and *Aedes chausseri* (7.1%) (Table 4.6).

Table 4.6: Mosquito Species Composition and Distribution Calculated in Percentages

Species	Nguruman		Kerio valley			Combined	
	Adult mosquitoes N(%)	Larvae N(%)	Adult mosquitoes N(%)	Larvae N(%)	p-values	Adult mosquitoes N(%)	Larvae N(%)
Aedes aegypti	536 (52.9)	68(76.4)	307 (45.3)	22(52.4)	0.047*	843 (49.85)	90 (68.7)
Aedes simpsoni	75 (7.4)	11(12.4)	175 (25.8)	11(26.2)	<0.001*	250 (14.78)	22 (16.8)
Aedes metallicus	274 (27)	8 (9)	5 (0.7)	0 (0.0)	<0.001*	279 (16.5)	8 (6.1)
Aedes africanus	0 (0)	0 (0.0)	28 (4.12)	3 (7.1)	<0.001*	28 (1.7)	3 (2.3)
Aedes stegomyia species	105 (10.3)	2 (2.2)	129 (19.02)	3 (7.1)	0.103	134 (7.92)	5 (3.8)
Aedes chausseri	6 (0.6)	0 (0.0)	34 (5.0)	3 (7.1)	<0.001*	40 (2.37)	3 (2.3)
Aedes hirsutus	9 (0.88)	0 (0.0)	0 (0)	0 (0.0)	0.01*	9 (0.53)	0 (0)
Aedes furcifer	3 (0.3)	0 (0.0)	0 (0)	0 (0.0)	0.083	3 (0.18)	0 (0)
Aedes tarsalis	4 (0.39)	0 (0.0)	0 (0)	0 (0.0)	0.045*	4 (0.24)	0 (0)
Aedes aedimorphus spp.	1 (0.1)	0 (0.0)	0 (0)	0 (0.0)	0.317	1 (0.06)	0 (0)
GRAND TOTAL	1013	89	678	42		1691	131

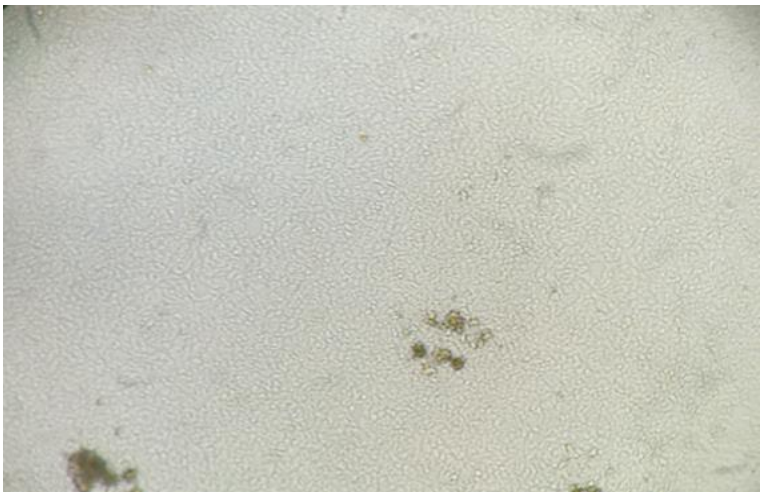
*Indicates a statistical significance at a 95% confidence level

The distribution of mosquito species differs significantly between Nguruman and Kerio Valley (P-value <0.001). The results showed significant differences in species distribution between Nguruman and Kerio Valley for the following species: *Aedes simpsoni* (p-value < 0.001), *Aedes metallicus* (p-value < 0.001), *Aedes africanus* (p-value < 0.001), *Aedes aegypti* (p value= 0.047). No significant difference was observed for *Aedes stegomyia spp.* (p = 0.103). Significant differences were also observed for Aedes chausseri (p-value < 0.001) and *Aedes tarsalis* (P-value < 0.001). *Aedes hirsutus* and *Aedes furcifer* showed substantial differences with small sample sizes and *Aedes aedimorphus spp*. exhibited no significant difference (p-value = 0.317) (Table 4.6).

4.5 Virus Isolation from *Aedes Stegomyia* Mosquitoes using Cell Culture

Mosquito samples collected were analysed in 1822 pools consisting of 7739 adult mosquitoes and 1444 mosquito larvae using cell culture. They were inoculated in both mammalian (Vero CCL-81 and E6) and mosquito cell lines (C6/36) to determine viral activity (cytopathic effects). The observed start of cytopathic effects (CPE) among the samples ranged from 3 to 12 days' post-infection. The images in figure 4.3 below displays wells showing negative and positive and CPE.

(A)



(B)

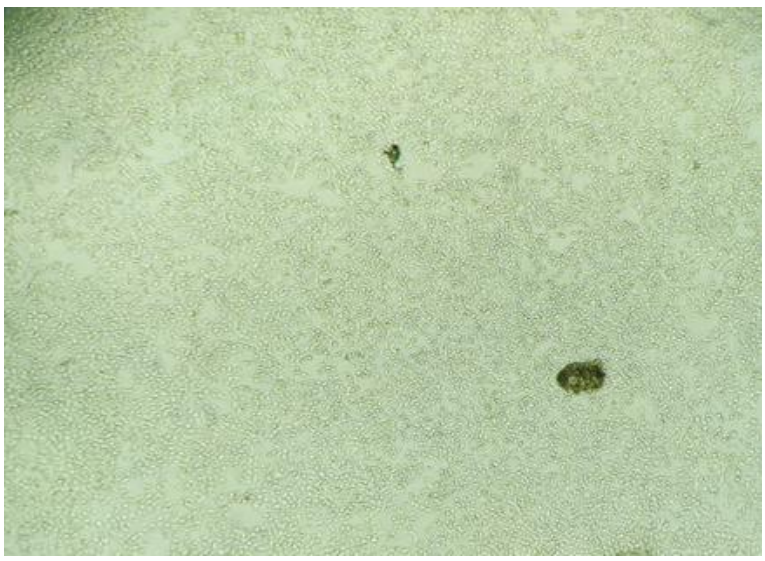


Figure 4.3: Wells showing (A) Negative for Cytopathic Effects (CPE) and (B) Positive for Cytopathic Effects (CPE) in Vero E6 Cells

Out of the total pools tested, 2.8% (51/1822) showed reproducible cytopathic effects (CPE) after two passages. Nguruman had 3.35% (37/1102) of pools showing cytopathic effect (CPE), while Kerio Valley had 1.94% (14/720) of pools with CPE (Figure 4.3). Nevertheless, there was no statistically significant difference in the proportion of pools exhibiting viral activity between the two sites (p-value = 0.081, 95% CI).

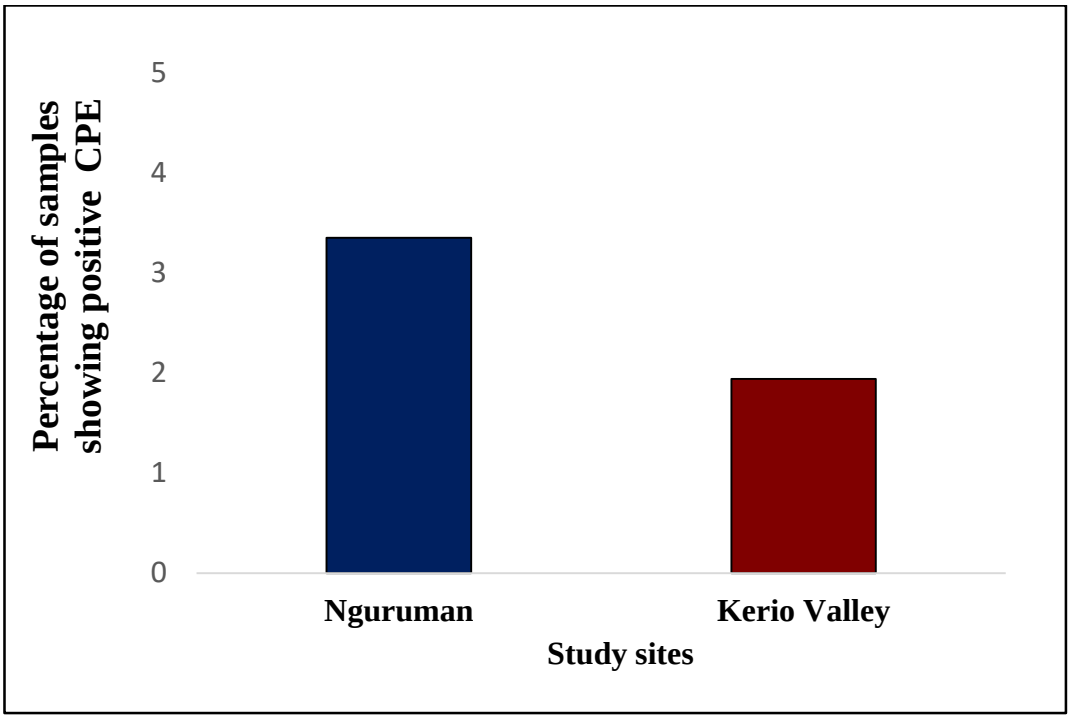


Figure 4.4: The Proportion of Samples Exhibiting Positive Viral Activity for Kerio Valley and Nguruman

The proportion with CPE also varied according to species. Overall, *Aedes aegypti* showed the highest percentage of viral activity (n=23/51, 45.09%) followed by *Aedes metallicus* (17.6%), *Aedes stegomyia* (17.6%) *Aedes simpsoni* (13.7%), *Aedes chausseri* (3.9%) *and Aedes africanus* (1.96%) (Figure 4.4).

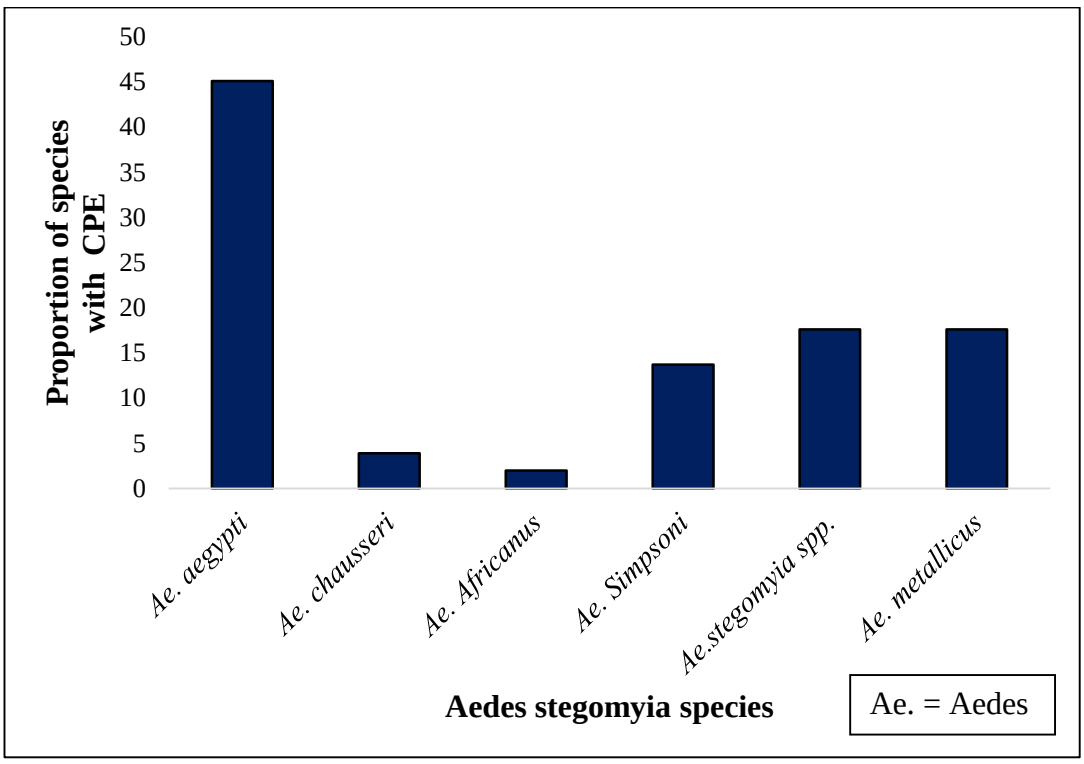


Figure 4.5: Proportion of Samples that Had Cytopathic Effects Categorized by Mosquito Species for Combined Study Sites

Aedes aegypti from Nguruman showed a significantly higher percentage (n=17, 33.3%) compared to Kerio Valley (n=6/51, 11.76%). Aedes chausseri had 11.8% in Nguruman as compared to 1.96% in Kerio Valley. *Aedes stegomyia spp*. had 9.8%, n=5 while Kerio Valley had 7.8%, n=4/51. *Aedes chausseri* and *Aedes simpsoni* only produced CPE in samples from Kerio Valley both at 1.96%,1/51. *Aedes Metallicus* produced CPE only in samples from Nguruman (17.6%, n= 9/51) (Figure 4.5).

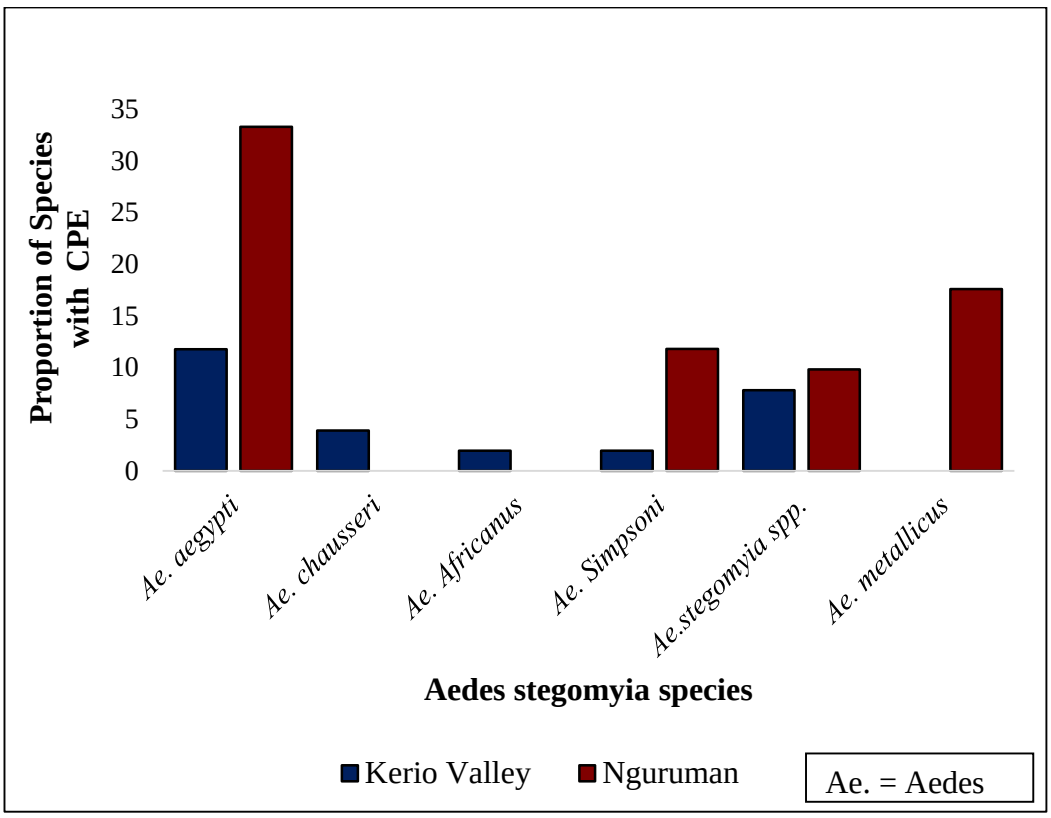
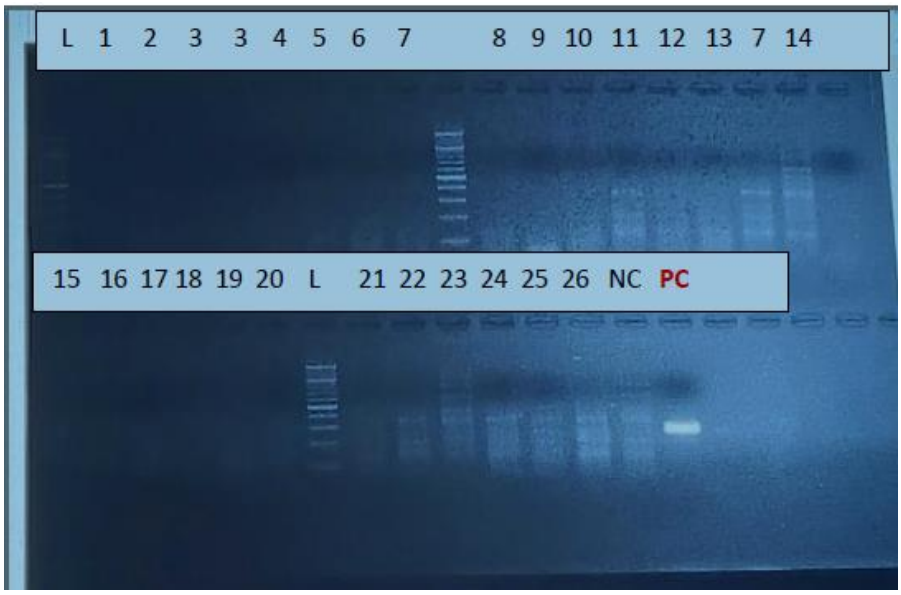


Figure 4.6: Proportion of Samples that had Cytopathic Effects Categorized by Mosquito Species for Individual Study Sites

4.6 Virus Detection Using RT- PCR

Out of the 51 samples tested, none had visible bands corresponding to the expected amplicon size for flavivirus (approximately 265bp), alphavirus (approximately 434bp) and Orthobunyavirus (approximately 363bp) families. The gel output results for the 51 samples showing no bands is shown in Figure 4.6 below.

(A)



(B)

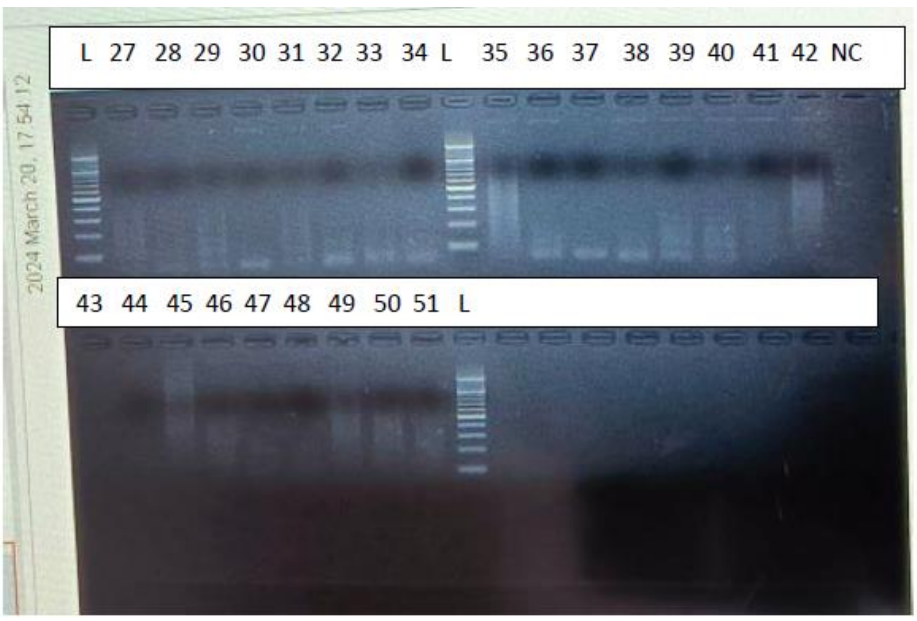


Figure 4.7: The Gel Output Results for the 51 Samples Showing No Bands for *Flavivirus* Family Using FU1 and CFD2

4.7 Virus Detection Using Sanger Sequencing

Since no amplicons were detected following RT-PCR and subsequent agarose gel electrophoresis analysis, Sanger sequencing was not performed. Consequently, downstream analyses such as genotyping and serotyping to determine the circulating genotypes of Yellow Fever virus and serotypes of Dengue virus were not conducted.

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

The emergence and re-emergence of arboviruses, including yellow fever and dengue viruses, have become a significant global public health issue with considerable socioeconomic impacts (Chepkorir *et al.,* 2019; Gubler, 2001; Ochieng *et al.,* 2013; Sang *et al.,* 2022). Most of these diseases in sub-Saharan Africa remain either undiagnosed or incorrectly diagnosed in remote, high-risk endemic regions that are challenging to access due to inadequate infrastructure, health facilities, or medical professionals (Kibathi *et al.,* 2024; Sang *et al.,* 2022). The objective of the study was to ascertain (i) the presence of yellow fever and dengue virus circulation in human serum samples utilising the Plaque Reduction Neutralisation Test (PRNT), (ii) the evidence of viral activity in Aedes Stegomyia mosquito populations, and (iii) the characterisation of viruses isolated from Aedes Stegomyia mosquitoes from the two study locations.

The elevated YFV exposure rates in Kerio Valley could be due to the extensive YFV vaccine implemented after the 1992/93 outbreak in Baringo and the ongoing routine YF immunisation for children aged 9 months (Reiter et al., 1998; Sanders *et al.,* 1998). Although routine immunization has the potential to provide long-term disease protection for up to three decades, yellow fever infections may still occur if sufficient herd immunity is not reached (Kibathi *et al.,* 2024; Chepkorir *et al.,* 2019). Additionally, the mosquito vectors responsible for YFV transmission, *Aedes keniensis* and *Aedes africanus* remain active in Baringo County and could potentially contribute to the spread of YFV (Johnson *et al.,* 1981; Karungu *et al.,* 2019; Reiter *et al.,* 1998). Even though no YFV outbreak has been recorded in Nguruman, this study indicates the possibility of low-level YFV circulation in the area. The economic activities including wildlife conservation, forest conservancies, and subsistence farming with

extensive irrigation, provide ideal mosquito breeding sites. The herders' regular migrations for pasture and water within these wooded conservancies enhance human-sylvatic mosquito interactions; thus, this encroachment into forest habitats creates both a reservoir and a transmission route for YFV (Pierson & Diamond, 2020). This finding is in agreement with studies by (Chepkorir *et al.*, 2019; Inziani *et al.*, 2020) where both studies recorded neutralizing antibodies against the yellow fever virus in northern and Western Kenya respectively. Another study reported a 6% yellow fever seroprevalence within specific healthcare facilities across Western Kenya (Kwallah *et al.,* 2015). Elsewhere, a community-based serosurvey done in Gambella Region, South-west Ethiopia found 2.9% seroprevalence of YFV-specific IgG in the blood samples collected (Asebe *et al.,* 2021).

Dengue-neutralizing antibodies were detected only in Nguruman. Dengue was first reported along the coastal cities of Kenya in 1982 (Johnson *et al.*, 1982). Since then, multiple positive cases have been reported, indicating a regional spread (Sutherland *et al.,* 2011). For instance, Kenya's coastal and northern regions reported dengue outbreaks in 2011(Konongoi *et al.,* 2016; Lutomiah *et al.,* 2013, 2016). The findings align with other studies that have documented positive dengue cases in Kenya (Chepkorir *et al.,* 2019; Inziani *et al.,* 2020; Ochieng *et al.,* 2015). The findings concur with Willcox *et al.,* 2018 where 0.4% dengue-neutralizing antibodies were detected in children from the Democratic Republic of Congo (DRC). Low Dengue endemicity has also been documented, confirming DENV in locations outside the initial outbreak zones (Chepkorir *et al.,* 2019; Langat *et al.,* 2020; Ochieng *et al.,* 2015). The dryland ecology in Nguruman coupled with climate change provides a conducive environment for *Aedes* mosquito vectors to thrive thus facilitating the transmission of dengue. These findings may imply the ongoing spread of dengue from the more endemic and prevalent Kenyan coast highlighting the need for continuous serosurveying to prevent potential outbreaks.

In both Nguruman and Kerio Valley, the seropositivity differed by age and gender. In Kerio Valley, neutralizing antibodies were substantially greater in females than in

males. This is consistent with the study's enrollment rates, which were likewise concentrated by gender, with more females than males enrolled. Compared to men, women are more likely to engage in hospital-seeking behaviors, which could account for the high enrollment of women. Men's low enrollment may be influenced by their primary occupations, which are farming and cattle grazing, as these occupations often keep them away from their homes. Additionally, men were more likely than women to have YFV neutralizing antibodies. This may be explained by the outside activities that males engage in, such as farming, pasture hunting, and livestock caring, which predispose them to infected mosquito bites. This finding concurs with a study done by (Chepkorir *et al.,* 2019) which found men to be likely exposed to yellow fever as compared to females.

A majority of the study participants (56.25%) were under the age of 25 years, with most being school-going children and adolescents. This reflects the typical age distribution of rural Kenyan populations and suggests that younger individuals are more likely to be accessible during sampling campaigns. In both Kerio Valley and Nguruman, individuals below 25 years exhibited the highest levels of neutralizing antibodies against Yellow Fever Virus (YFV), indicating recent or ongoing exposure. The elevated seropositivity in this age group may be attributed to increased outdoor activities, such as playing or walking long distances to school, which enhance their contact with vector habitats particularly forested or bushy environments where Aedes mosquitoes are prevalent. Additionally, lower levels of prior immunity in this age group may make them more susceptible to initial infections, resulting in detectable antibody responses (Kibathi *et al.,* 2024). On the contrary, the highest levels of dengue virus (DENV) neutralizing antibodies were observed in the 26–45 years' age group. This finding suggests that adults in this age bracket may experience more frequent or cumulative exposure to DENV over time, possibly due to occupational factors such as farming, herding, or trade-related travel, which increase the likelihood of contact with *Aedes aegypti* the primary urban and peri-urban vector for DENV. Unlike children, adults in this age group may also have greater mobility across regions with varying levels of dengue virus circulation, contributing to higher seroprevalence.

The higher YFV seropositivity among younger individuals in both sites may also indicate that yellow fever virus transmission in these regions is enzootic, occurring silently and potentially involving non-human primate reservoirs and sylvatic Aedes vectors (Agha *et al.,* 2017a). In such settings, periodic spillover to humans, especially those living or schooling near forested areas can occur without triggering noticeable outbreaks, especially if infections are asymptomatic or mild. The age-related patterns of antibody distribution emphasize the importance of targeted public health interventions, including risk communication, vector control, and potentially vaccination campaigns for high-risk groups and reinforce the need for strengthened arboviral surveillance, especially in ecologically diverse regions like Kerio Valley and Nguruman where sylvatic and zoonotic transmission cycles may be sustained.

Mosquitoes play a significant role in the transmission and maintenance of arboviruses within the East African region and have served as effective vectors for diseases such as dengue, yellow fever, and chikungunya (Bisimwa *et al.,* 2016; Ochieng *et al.,* 2013; Sang *et al.,* 2022; Tigoi *et al.,* 2015; Weaver & Reisen, 2010). Their adaptability enables them to thrive under various environmental conditions of highly populated urban areas with very high human-mosquito interactions (Gould & Higgs, 2009; Tchouassi *et al.,* 2022). The seasonal fluctuation affects the outbreaks; hence, the same requires continued surveillance and studies to enhance the vector control approach. Addressing the role that mosquitoes play in public health in Kenya is vital. The study shows that a diverse range of mosquitoes exist in Nguruman and Kerio Valley with *Aedes aegypti* being the most predominant species followed by *Aedes metallicus*, *Aedes simpsoni* and *Aedes africanus*. Previous research conducted in Kenya has demonstrated that these arboviral vectors are found in large numbers and are widely distributed across Kenya (Lutomiah *et al.,* 2016; Ochieng *et al.,* 2013). *Ae. africanus* was previously documented in elevated forests in Baringo during the yellow fever epidemic of 1992–1993 (Reiter *et al.,* 1998).

Aedes aegypti which is widely distributed across Kenya is a well-known vector of multiple arboviruses that are significant for public health such as the Zika virus (ZKV),

chikungunya virus, dengue virus (DENV), and yellow fever virus (YFV) (Eastwood *et al.,* 2020; Kaboré *et al.,* 2023; Karungu *et al.,* 2019; L. Konongoi *et al.,* 2016; Konongoi *et al.,* 2018; Lutomiah *et al.,* 2013, 2016; Uwishema *et al.,* 2022). Several studies have recorded *Aedes aegypti* as the predominant species of the collected mosquitoes (Kaboré *et al.,* 2023; Karisa *et al.,* 2021). The results further concur with a study on molecular detection of arboviruses in yellow fever mosquitoes in Tanzania where *Aedes aegypti* was recorded as the predominant species followed by *Aedes africanus* (Bisimwa *et al.,* 2016). The high abundance of *Aedes* species in Kerio Valley and Nguruman can be attributed to the characteristics of the terrain, soil composition, vegetation cover, and rainfall, which may provide suitable breeding and resting sites for vectors (Karungu *et al.,* 2019; Lutomiah *et al.,* 2013). The natural environment can be altered by human activity, creating new ecological niches that could force mosquito species to adapt or become extinct (Kaboré *et al.,* 2023)

The study did not isolate dengue and yellow fever viruses from *Aedes stegomyia* collected from Nguruman and Kerio Valley, Kenya. The absence does not imply that these viruses are non-existent in the region; rather, it may indicate low infection rates or that the viruses were not in circulation during the sampling period. However, the finding is consistent with other studies. For instance (Iwashita *et al.,* 2018) in their study on mosquito arbovirus survey in Kenya whereby no human-related arboviruses such as dengue, yellow fever, West Nile and O'nyong-nyong viruses were isolated from mosquitoes. The low viral load in mosquitoes may contribute to the relatively poor detection of YFV and DENV, making detection by RT-PCR or direct isolation extremely difficult, especially without an outbreak (Oyono *et al.,* 2022). A further constraint is the limited sample size, as well as the restricted number of mosquito species. More extensive research is required to yield a more precise assessment of arbovirus prevalence. Moreover, the abundance of *Aedes aegypti,* one of the most potent arboviral vectors in the human environment, was lower than expected. This mosquito is believed to have originated in Africa and to have been disseminated to other continents, including Asia and South America, via maritime trade (Iwashita *et al.,* 2018). The adaptability of this mosquito to urban environments on various

continents has rendered DENV transmission by *Ae. aegypti* a significant danger to human health. This study found no pools positive for dengue virus among the *Aedes aegypti* samples.

5.1.2 Limitations and Future Studies

Although this study is deemed successful, its limitations are acknowledged. First, the study only focused on one dengue virus serotype (DENV-2) suggesting that further research on the genetic variety of DENV is necessary to determine the exact prevalence of dengue and the distribution of its serotypes in the study area. Secondly, a larger mosquito sample size was required to increase the likelihood of isolating different viruses. Thirdly, it is important to note that while the study documented the presence of mosquito vectors, it did not succeed in isolating the dengue and yellow fever viruses from *Aedes Stegomyia* mosquitoes collected in these regions. This limitation suggests that while the conditions for arbovirus transmission may be present, additional research and surveillance studies are required to confirm the active transmission of these viruses within mosquitoes in the area.

5.3 Conclusions

1. The study's findings indicate the circulation of yellow fever (YFV) and dengue viruses (DENV) within human populations in Nguruman and Kerio Valley. Both of these viruses represent serious public health threats, particularly in regions where surveillance and diagnostic capabilities may be limited. The presence of these viruses in these areas raises concerns about the potential for under-recognized or misdiagnosed cases, which could lead to delayed interventions and increased morbidity.
2. The study demonstrated detectable viral activity (shown by the reproducible cytopathic effects on Vero cells) in *Aedes Stegomyia* mosquitoes collected from both Nguruman and Kerio Valley, Kenya. There was also presence of *Aedes stegomyia* species in Nguruman and Kerio Valley – known vectors for

DENV and YFV with *Aedes aegypti* being the predominant species in both sites.

3. Dengue and yellow fever viruses were not isolated from *Aedes stegomyia* mosquitoes collected in Nguruman and Kerio Valley, despite the presence of mosquito vectors for these and other arboviral diseases.

5.4 Recommendations

1. The presence of YFV and DENV antibodies in both sites suggests strengthening surveillance and diagnostics for arboviral diseases.
2. Enhance mosquito surveillance to monitor viral activity for early outbreak detection and timely vector control.
3. Incorporate advanced methods like metagenomic testing into mosquito surveillance to improve the detection of low-level DENV and YFV in mosquitoes for effective serotyping and genotyping.

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APPENDICES

Appendix I: Informed Consent Agreement

What is the study called? Epidemiologic Assessment of Risk of Yellow Fever and Dengue Transmission and Outbreaks in Kenya.

What is this study about? We are interested in finding out the extent to which individuals living in this area and community are exposed to a range of arboviruses based on the surrounding environment and interaction with vectors, livestock herds and wild rodents moving within this environment. We will use the samples collected from you to determine if you have been exposed to these arboviruses in comparison to other households living in other locations. We want to draw a small amount of blood (about a spoonful) and test it in the lab to see if we can find antibodies to arboviruses like Rift Valley Fever, Dengue, Chikungunya, Zika and others which make people very sick. This will be evidence that you have been infected recently or sometime back. It will tell us that the viruses are present here, information that is important in planning disease prevention by the government.

Who is running the study? Dr Rosemary Sang and a team of experts and scientists from, the Ministry of Health, Ministry of Livestock, University of Bonn in Germany and ICIPE are running the study. Your sample and record data will be kept by Dr Rosemary Sang on behalf of the research team.

Do I have to participate: Your participation in this study is voluntary? There is no penalty for refusing to participate. If you start the study you (your child) may discontinue your (your child's) participation at any time. The principal investigators and co-investigators from the research team may also decide to withdraw you (your child) from the study if we are unable to obtain a blood sample from you (your child).

What will happen to me if I participate in the study? We will ask you some questions about yourself, where you live, your occupation, your current illness including symptoms if you have previously suffered a major illness that could be arbovirus infection, where you have lived before and when, where you have travelled and your ownership and association with livestock. Then about a tablespoon of blood will be taken from a vein in your arm. The blood will be put into small tubes so we can test for RVFV antibodies to determine if you have evidence of previous RVF and other arbovirus infections) exposure. If you also accept, the remaining sample will be used later to test for previous exposure to any other arboviruses that could mimic malaria but may be arbovirus infection that we detect in the vectors in your surroundings. We will not test for HIV or any other virus that we have not discussed with you and agreed to. We will only test for arboviruses.

Are there any risks if I participate in the study? There is the possibility of mild discomfort, bruising and very rarely infection at the site where the blood is taken. But,

should you (your child) be injured as a direct result of participating in this research project, you (your child) will be provided medical care, at no cost to you (your child), for that injury at the nearest government health facility. You (your child) will not receive any injury compensation, only medical care. You (your child) should also understand that this is not a waiver or release of your (your child's) legal rights. If in doubt you should discuss this issue thoroughly with the principal investigator before you (your child) enrol in this study.

Are there any benefits from the study? The study can lead to a better understanding of the arboviruses that are affecting you in this area and community, the level of risk of your community and household and how you and your household/community can be protected. The MOH and livestock will be supported by the data to develop prevention plans

Will there be any compensation for being in the study? There is no compensation to volunteers for their participation.

How long does the study last? This study requires the completion of a questionnaire and one blood draw. The questionnaire and blood draw will take about 30 minutes. There will be no further sample-taking after this.

Who can participate in this study? Anyone can participate in the study if they live within the selected village or cluster if they have an illness that the clinician at your hospital has identified to fit the cases we are looking to enrol, aged 5 years and above and if willing and consent to participate.

Who will be able to see my information or lab results? Any information about you (or your child's) will remain confidential. Only the people involved in the study will be able to see your information. We will keep all files in locked cabinets when they are not in use, and all blood stored in locked freezers. Your (your child's) name will not be used in any report resulting from this study or in the sample vial that will be used in the laboratory. Only a unique code (sample ID number) will be used. Any report from this study will refer to you/your child only by a study identification number and not by a name. All blood samples collected will be labelled with a study identification number; no names will be used.

What will happen to my blood? Your (your child's) blood will be tested for antibodies to a range of arboviruses that we suspected to be circulating in this area and other arboviruses detected in vectors in your surroundings. A sample of your blood will be kept frozen in case we want to do more testing to confirm our findings in the future. These samples will be labelled with only your study number. They will be secured in freezers at KEMRI and ICIPE emerging infectious diseases laboratory and only study investigators and their authorized staff will have access. All safeguards ensuring privacy and confidentiality that are in place during this study period will also continue to be in place for the long-term storage of samples.

If we do need to use the stored blood in the future for any study other than arbovirus testing, we will first get permission from you and also from the Ethical Review Committee.

Who can I contact about the study or my rights as a volunteer in this research study? If during this study, you have questions concerning the nature of the research or you believe you have sustained a research-related injury, you should contact:

Who can I contact if I need information on the conduct of the study?

If you have any questions you or your parent should contact:

Dr Rosemary Sang

Kenya Medical Research Institute

P. O. Box 54628, NAIROBI

KENYA

Phone: +254 02 722 541

Cell phone: Tel. 0722 759492

Who should I contact if I have questions about my rights as a volunteer in this research study: If you have any questions on your rights as a volunteer, you or your parent should contact me;

The Secretary, Ethical Review Unit c/o Kenya Medical Research Institute

P.O. Box 54840, Nairobi, Kenya

Tel. 254-20-2722541

IF THERE IS ANY PORTION OF THIS CONSENT AGREEMENT THAT YOU DO NOT UNDERSTAND, PLEASE TALK TO SOMEONE ON THE STUDY TEAM BEFORE SIGNING.

Subject's or Parent/Guardian's Signature: _____Date: _____

Permanent Address: _____

Witness's Name: _____

Witness's Signature: _____ Date: _____

Study Number: _____

Thumbprint of Volunteer or Volunteer's Parent/Guardian if Unable to Sign

Person Administering Consent:

Name: _____

Signature: _____

Appendix II: Assent Form for Individuals 5 Through 17 Years of Age

What is the study called?

Epidemiologic Assessment of Risk of Yellow Fever and Dengue Transmission and Outbreaks in Kenya.

What is this study about? We are interested in finding out the extent to which individuals living in this area and community are exposed to a range of arboviruses based on the surrounding environment and interaction with vectors livestock herds and wild rodents moving within this environment. We will use the samples collected from your child to determine if you have been exposed to arboviruses in comparison to other households living in other locations. We want to draw a small amount of blood (about a spoonful) and test it in the lab to see if we can find antibodies to arboviruses like Rift Valley Fever, Dengue, Chikungunya, Zika and others which make people very sick. This will be evidence that you have been infected recently and sometime back. It will tell us that the viruses are present here, information that is important in planning disease prevention by the government.

Who is running the study? The study is being run by Dr Rosemary Sang and a team of experts and scientists from, the Ministry of Health, Ministry of Livestock, University of Bonn in Germany and ICIPE. Your sample and record data will be kept by Dr Rosemary Sang on behalf of the research team.

Do I have to be in the study? Your participation in this study is voluntary. There is no penalty for refusing to participate. If you start the study you (your child) may discontinue your (your child's) participation at any time. The principal investigators and co-investigators from the research team may also decide to withdraw you (your child) from the study if we are unable to obtain a blood sample from you (your child).

What will happen to me if I participate in the study? You and the adult you are with will be asked some questions about yourself, where you live, your occupation, your current illness including symptoms if you have previously suffered a major illness that could be arbovirus infection, where you have lived before and when, where you have travelled and your ownership and association with livestock. Then about a tablespoon of blood will be taken from a vein in your arm. The blood will be put into small tubes so we can test for RVFV antibodies to determine if you have evidence of previous testing for arbovirus exposure. If you also accept, the remaining sample will be used later to test for previous exposure to any other arboviruses that could mimic malaria but may be arbovirus infection that we detect in the vectors in your surroundings. We will not test for HIV or any other virus that we have not discussed with you and agreed on.

Are there any risks if I participate in the study? There is the possibility of mild discomfort, bruising and very rarely infection at the site where the blood is taken.

While everything has been done to ensure your safety in the process of taking your sample, should your child be injured as a direct result of participating in this research project, you will be provided medical care, at no cost to you or the adult you are with for that injury at the nearest government hospital in the area. You or the adult you are with will not receive any injury compensation, only medical care. You and the adult should also understand that this is not a waiver or release of your legal rights. If in doubt you should discuss this issue thoroughly with the principal investigator before you enrol in this study.

Are there any benefits from participating in the study? The study can lead to a better understanding of the way the arboviruses move in your environment the source and level of risk of your community and household and how you and your household/community can be protected. The MOH and livestock will be supported by the data to develop RVFV prevention plans.

Will I get anything for being in the study? No, you do not receive anything for being in the study.

How long does the study last? This study requires only the completion of a questionnaire and/or one blood draw. The questionnaire and blood draw will take about 30 minutes each time.

Who can be in this study? Anyone can participate in the study if they live within the selected village, or cluster, have been seen at the health facility participating in this study and identified by the clinician to fit the case required for this study, aged 5 years and above and willing and consent to participate.

Who will be able to see my information or lab results? Only the scientific staff involved in the study will see your data. Your name will be removed from everything that anyone else sees. Any information about you will remain confidential. Only the people involved in the study will be able to see your information. We will keep all files in locked cabinets when they are not in use, and all blood stored in locked freezers. Your name will not be used in any report resulting from this study. Any report from this study will refer to you by a study identification number and not by a name. All blood samples collected will be labelled with a study identification number; no names will be used.

What will happen to my blood? Your blood will be tested for antibodies to arboviruses that make people sick or other arboviruses detected in vectors in your surroundings. A sample of your blood will be kept frozen in case we want to do more testing to confirm our findings in the future. These samples will be labelled with only your study number. They will be secured in freezers at KEMRI and ICIPE facilities and only study investigators and their authorized staff will have access. All safeguards ensuring privacy and confidentiality that are in place during this study period will also continue to be in place for the long-term storage of samples.

If we do need to use the stored blood in the future, we will first get permission from you and also from the Kenya National Ethical Review Committee.

Who can I contact if I need information on the conduct of the study?

If you have any questions you or your parent should contact:

Dr Rosemary Sang

Kenya Medical Research Institute

P. O. Box 54628, NAIROBI

KENYA

Phone: +254 02 722 541

Cell phone: Tel. 0722 759492

Who should I contact if I have questions about my rights as a volunteer in this research study? If you have any questions about your rights as a volunteer, you or your parent should contact:

The Secretary, National Ethical Review Unit

C/o Kenya Medical Research Institute

P.O. Box 54840, Nairobi, Kenya

Tel. 254-20-2722541

IF THERE IS ANY PORTION OF THIS CONSENT AGREEMENT THAT YOU DO NOT UNDERSTAND, PLEASE ASK THE STUDY TEAM BEFORE SIGNING.

Subject's or Guardian's Signature: _____ Date: _____

Permanent Address: _____

Witness's Name: _____

(Must be literate in case of an illiterate participant)

Witness's Signature: _____ Date: _____

Study Number: _____

Thumbprint of Volunteer or Volunteer's Parent/Guardian if Unable to Sign

Person Administering Consent:

Name: _____

Signature: _____

Appendix III: Demographic Data Collection Sheet

Interviewer _____

Study Number _____

CLINICAL DEMOGRAPHIC DATA SHEET

Date of sample Collection: _____ (dd/mon/yr)

Sex: Male Female Age: _____years

Do you (your child) have any of the following symptoms?

	Yes	No	Uncertain
Fever	Temperature:		
Headache			
Joint pain			
Pain around the eyes			
Backache			
General body weakness			
Abdominal pains			
Bleeding from nose			
Bleeding from other sites (specify)			
Other symptoms (specify)			

How many days have you (your child) been sick?_____

Where is your (your child's) current residence:

Village: _____ Sub-County: _____ County: _____

How long have you (your child) been living in this County? _____years _____months

How long have you (your child) been living in this village? _____years _____months

Where did you live before you came here? _____

Village of Residence _____

Sub-County _____

Out of the County, Where? _____

How many times have you (your child) changed residence in the last 5 years: _____?

Where did you move from? 1. _____

2. _____

3. _____

If adult: What is your occupation: _____

If a child: Where do you go to school: _____

Do you have contact with any of the following species of animals?

1. Bats
2. Guinea fowl
3. Ducks
4. Chickens
5. Other birds (Name)_____
6. Goats
7. Cows
8. Donkeys
9. Sheep
10. Camels
11. Monkeys
12. Other Animals: _____

For each species checked above:

1) List the species

2) Describe the contact, e.g., trapping, farming, slaughter, food preparation, veterinary work, casual contact (e.g., a neighbour keeps chickens, there is a slaughterhouse nearby), eating raw fowl products or drinking blood

Do you recall any time you were severely ill with bleeding symptoms (from nose, mouth, abdomen etc) _____?

Approximately how long ago? _____

What do you think was the cause? _____

Appendix IV: Scientific and Ethical Review Unit Approval Letter

In Search of Better Health

KENYA MEDICAL RESEARCH INSTITUTE

OFFICE OF THE DIRECTOR RESEARCH & DEVELOPMENT

Tell: +254 020 2722541, 2713349,
0722 205 901, 0733 400 003

P.O. Box 54840-00200, Nairobi
Email: ddrt@kemri.go.ke
Website: www.kemri.go.ke

KEMRI/RD/22 **March 24, 2023**

TO: **MERCY HOKAH KIBATHI,**
PRINCIPAL INVESTIGATOR.

THROUGH: **THE DEPUTY DIRECTOR, CVR,**
NAIROBI.

Dear Sir,

RE: **KEMRI/SERU/CVR/013/4664 *(RESUBMISSION OF INITIAL):* YELLOW FEVER AND DENGUE VIRUS CIRCULATION AMONG HUMAN AND MOSQUITO POPULATIONS IN NGURUMAN AND KERIO VALLEY, KENYA**

Reference is made to your letter responding to KEMRI SERU comments. The KEMRI Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised study documents on March 14, 2023;

1. Response to issues raised by SERU.
2. The revised protocol, version 2, March 14, 2023.
3. Ethics certificates for the Investigators.
4. CVs for the investigators.
5. Permission letter and ICF for SSC 2787

This is to inform you that the Committee notes that the issues raised during 332nd Committee C meeting of the KEMRI Scientific Ethics Review Unit (SERU) held on **February 23, 2023** have been adequately addressed.

Consequently, the study is **granted approval** for implementation effective this day, **March 24, 2023** for a period of **one (1) year**. Please note that authorization to conduct this study will automatically expire on **March 23, 2024.** If you plan to continue with data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **February 09, 2024.**

Please note that only approved documents including (informed consents, study instruments, Material Transfer Agreement) will be used. 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. 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.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Yours faithfully,

ENOCK KEBENEI,
THE ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT

Appendix V: NACOSTI Approval Letter

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Ref No: **433213**

Date of Issue: **26/April/2023**

RESEARCH LICENSE

This is to Certify that Ms.. Mercy Hokah Kibathi of Jomo Kenyatta University of Agriculture and Technology, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Baringo, Kajiado on the topic: Yellow Fever and Dengue Virus Circulation among human and mosquito populations in Nguruman and Kerio Valley, Kenya for the period ending : 26/April/2024.

License No: **NACOSTI/P/23/25147**

433213

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

See overleaf for conditions

Appendix VI: Permission Letter to Use the Study Samples

8th March 2023

The Head

KEMRI Scientific and Ethics Review Unit (SERU)

KEMRI, NAIROBI

Yellow Fever and Dengue Virus Circulation among human and mosquito populations in Nguruman and Kerio Valley, Kenya. (SERU 4664)

This is to confirm that Ms Kibathi Mercy Hoka has permission to use study samples collected under our project tilted "*Epidemiologic Assessment of the Risk of Yellow Fever and Dengue Transmission and Outbreaks in Kenya*" (SSC 2787). Ms Kibathi is a graduate student working towards her MSc through her proposal titled *Yellow Fever and Dengue Virus Circulation among human and mosquito populations in Nguruman and Kerio Valley, Kenya (SERU 4664),* which is nested in our larger continuing proposal. Kindly accord her the necessary support.

Rosemary Sang (PI)

Appendix VII: Publication

frontiers | Frontiers in Virology

TYPE Original Research
PUBLISHED 24 September 2024
DOI 10.3389/fviro.2024.1459021

OPEN ACCESS

EDITED BY
Glenn Andrew Marsh,
Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia

REVIEWED BY
Dana Mitzel,
United States Department of Agriculture, United States
Maia Kavanagh Williamson,
LifeArc, United Kingdom

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Mercy Hokah Kibathi
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Rosemary Sang
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RECEIVED 03 July 2024
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PUBLISHED 24 September 2024

CITATION
Kibathi MH, Chepkorir E, Mabeya SN, Tchouassi DP and Sang R (2024) Seroprevalence of dengue, yellow fever, and related flaviviruses among the rural human population in Nguruman and Kerio Valley, Kenya.
Front. Virol. 4:1459021.
doi: 10.3389/fviro.2024.1459021

Seroprevalence of dengue, yellow fever, and related flaviviruses among the rural human population in Nguruman and Kerio Valley, Kenya

Mercy Hokah Kibathi[1,2*], Edith Chepkorir[3], Sepha Nyatichi Mabeya[2], David P. Tchouassi[1] and Rosemary Sang[1*]

[1]Human Health Division, International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya, [2]Department of Medical Microbiology, Jomo Kenyatta University of Science and Technology (JKUAT), Nairobi, Kenya, [3]Centre for Virus Research, Kenya Medical Research Institute (KEMRI), Nairobi, Kenya

Background: Yellow fever virus (YFV) and dengue virus (DENV) are among the major re-emerging arboviruses that pose a significant threat to public health. Their associated burden and prevalence can be substantially underestimated due to insufficient surveillance and inadequate diagnosis. This study aimed to determine evidence of dengue, yellow and related flaviviruses circulation among the rural human populations residing in Nguruman (Kajiado County) and Kerio Valley (Baringo County), two dryland ecosystems in the Kenyan Rift Valley.

Methods: Serum samples obtained from febrile patients between 5 and 85 years through a hospital-based cross-sectional survey from July 2020 – May 2023, were screened for neutralizing antibodies to YFV, DENV-2 and related flaviviruses, West Nile virus (WNV) and Zika virus (ZIKV) via Plaque reduction neutralization test (PRNT). The study sites and important demographic characteristics were obtained using a structural questionnaire and the data analyzed and seroprevalence compared. A multinomial logistic regression model was done to predict risk for each of the most prevalent viruses with covariates; age, gender, and occupation.

Results: Overall, 54.5% (50.1–59.0% 95% confidence interval (CI) of the samples tested positive for at least one of the four Flaviviruses. The percentage was significantly higher in Kerio Valley (64.34%, 184/286) than in Nguruman (40.2%, 78/194) (P<0.0001). YFV had the highest prevalence, followed by WNV (16.25%), ZIKV (5.2%), and DENV-2 (1%). Kerio Valley had a significantly higher YFV seroprevalence (51%) than Nguruman (6%) (P<0.0001), while DENV-2 was observed only in Nguruman with a low seropositivity of 2%. In contrast to Nguruman, where seropositivity rates were higher in males at 47.47% (P=0.049), in Kerio Valley, females showed considerably higher viral seropositivity at 60.82% than males (P<0001).

Conclusion: The study suggests that there is significant circulation of Flaviviruses in both regions, posing a public health risk, that could potentially contribute to clinical disease. However, seropositivity rates vary for each specific site.

Furthermore, there could be a risk of YFV, WNV, and ZIKV transmission in both sites with DENV transmission specifically noted in Nguruman. The study findings inform direct cost-effective actions (such as YF vaccines) and precise surveillance data of vector populations for improved disease risk prediction.

KEYWORDS

arbovirus surveillance, flaviviruses, yellow fever virus, dengue virus, plaque reduction neutralization test, Zika virus, West Nile virus, seroprevalence

1 Introduction

Dengue virus (DENV) and yellow fever virus (YFV) belong to the family *Flaviviridae* and genus *Flavivirus* and cause significant morbidity, mortality, and economic burden, especially in Sub-Saharan Africa (1–4). They are mostly endemic in tropical and subtropical regions (5). These illnesses share a similar ecological niche, with nonhuman primates serving as reservoir hosts, and are largely transmitted by *Aedes* subgenus *Stegomyia* mosquitoes (6, 7).

Dengue virus is the world's fastest-spreading arbovirus with nearly half of the global population (about 4 billion people) now at risk of contracting dengue fever, and more particularly in tropical and subtropical regions (3). An unanticipated surge in dengue infections coupled with continuous transmission since the start of 2023 has led to a record high of over 6.5 million illnesses and over 7300 dengue-related deaths recorded, indicating a widening epidemiological scope (3, 6). Dengue virus occurs in four closely related, but distinct serotypes (DENV 1 – 4) (8, 9), each causing dengue fever. More than 80% of human dengue cases manifest as minor acute flu-like diseases, with around 5–10% of human dengue cases developing into severe dengue, which is characterized by dengue shock syndrome and hemorrhagic fever (3, 10, 11).

Dengue fever virus was first detected in Kenya during an outbreak in the coastal region of Mombasa, Kilifi, and Malindi in 1982 (12) followed by another outbreak in Mandera, North Eastern Kenya, close to three decades later in 2011, that subsequently spread to the Kenyan coast (13–15). Kenya has documented cases of all four dengue viral serotypes 1 – 4, with the dengue 2 virus being the most common serotype (15, 16). This geographic dengue outbreak trend in Kenya is of concern as it shows the potential for further transmission and spread (7).

Yellow fever virus was first detected in Kenya in Kerio Valley, Baringo County in 1992-93 (17, 18). The bulk of YF cases occur in West and Sub-Saharan Africa, where 33 nations and an estimated more than 900 million people live in YFV endemic regions, accounting for roughly 90% of total global infections that result in 30,000 to 200,000 cases each year (19, 20). The recent YFV outbreak in Central and East Africa occurred in 2015 in urban areas of Angola's Luanda Province, resulting in over 7000 cases and 500 deaths before spreading to Kinshasa (DRC) by July 2016 (7, 21). Kenya has been categorized as being at high risk for YF transmission and outbreaks according to the Eliminate Yellow Fever Epidemics Strategy (EYE) by the World Health Organization (22). Furthermore, the central part of Kenya, Isiolo Counties recorded a total of 53 probable yellow fever cases and six mortalities in March 2022. From suspected cases, six were confirmed using Enzyme-linked immunoassay (ELISA) and Plaque reduction neutralization assay, with reverse transcription polymerase chain reaction (RT-PCR) confirming two cases, suggesting possible cases of yellow fever (23). Given the recent recurring YFV outbreaks in the neighboring nations of Uganda, South Sudan, and Ethiopia (2, 24–27), there is potential for cross-border spill-over via migration of infected non-human primates, people, or mosquitoes.

The related *Flavivirus*, West Nile virus (WNV) a zoonotic mosquito-borne virus initially isolated in Uganda in 1947 is currently re-emerging with a geographic range and frequency continually expanding (2, 28, 29). Zika virus (ZIKV), on the other hand, was discovered in the Zika Forest, Uganda in 1947 after being isolated from a febrile monkey. It was later found in *Aedes africanus* mosquitoes collected from the same forest, and human cases were found there in 1962–1963 (7, 30). The first records of the ZIKV virus and humans date back to 1954, when three cases in Nigeria with fever and jaundice were found (31). Ever since, human serosurveillance studies have indicated that the Zika virus is present in Asia, Oceania, and Africa (30–32). According to a recent investigation of samples collected during the dengue outbreak in 2013, ZIKV co-circulated in Kenya (33). In addition, another study discovered that up to 7% of Northern Kenyans had neutralizing anti-ZIKV antibodies (2). Following insufficient surveillance, the actual ZIKV burden in Kenya remains poorly known.

The increasing frequency and geographic expansion of these arboviruses in recent decades in Africa remains a concerning threat (21). These increasing trends of cases connected to the rapidly increasing human population, fast-expanding urbanization without sufficient sanitary infrastructure, increased international travel, deforestation, and climate change call for public health intervention (4, 34).

Abbreviations: CI, Confidence Interval; DENV, Dengue Virus; ELISA, Enzyme-Linked Immunoassay; OR, Odds ratio; PRNT, Plaque Reduction Neutralization Test; RT-PCR, Reverse Transcription Polymerase Chain Reaction; WHO, World Health Organisation; WNV, West Nile virus; YFV, Yellow fever virus; ZIKV, Zika Virus.

Epidemiologic and entomological data on the prevalence and distribution of these *Flaviviruses* in Nguruman and Kerio Valley are scarce. The low level of clinical awareness and inadequate diagnostic capabilities limit disease recognition and detection of cases at the facility level (7). This problem is made worse by the co-existence of other endemic diseases like leptospirosis, brucellosis, and malaria and by the limited capacity for differential diagnoses in facilities with inadequate health infrastructure. Consequently, the extent of exposure and the prevalence of these serious *Flavivirus* infections in these areas are still poorly understood. Thus, this study sought to determine evidence of dengue, yellow fever, and related Flaviviruses among the rural human population residing in Nguruman and Kerio Valley, Kenya.

2 Materials and methods

2.1 Ethical approval

The study was granted approval by the National Commission for Science, Technology & Innovation under Research license number NACOSTI/P/23/25147 and the Scientific Ethics and Review Unit (SERU) of the Kenya Medical Research Institute (KEMRI) under protocol number KEMRI/SERU/CVR/013/4664. Written informed consent was given by each adult participant, and minors' or children's assent was obtained. In the study, only those who gave their assent or consent were included.

2.2 Study design, sites, and population

The study was a cross-sectional hospital-based serosurvey that leveraged an acute febrile illness surveillance sampling of patients presenting with febrile illness (a body temperature >37.5 degrees Celsius, confirmed negative for malaria, typhoid and brucellosis) who were identified and sampled by local clinical staff at the Nguruman Health Centre in Nguruman town, and in Marigat Sub-County hospital, in Kerio Valley (Figure 1). The human population in both Nguruman and Kerio Valley are mostly pastoralists and occasionally farmers. In 2019, 666, 763 people lived in Baringo and 1,117,840 people in Kajiado (35). Both Nguruman and Kerio Valley have a similar dryland ecosystem occupied by largely pastoralist communities, diverse livestock and wildlife species with past evidence of arboviral circulation (36–38). Low-lying plains and seasonal rivers provide grazing land, water for irrigation, food for animals and wildlife, and a means of subsistence for the human population. Both sites have dry African savannah terrain, with low-lying Acacia plants, discontinuous trees and long grasses predominating. While Kerio Valley in Baringo County is around 250 kilometers northwest of Nairobi, Nguruman in Kajiado County is situated in southern Kenya and borders Tanzania (Figure 1). Unlike Nguruman which has never experienced a YFV outbreak nor YFV vaccination, Kerio Valley experienced an outbreak of YFV in 1992/93 which was brought under control by mass vaccination with the subsequent institution of routine YF immunization for all children at 9 months in Kerio Valley to avert

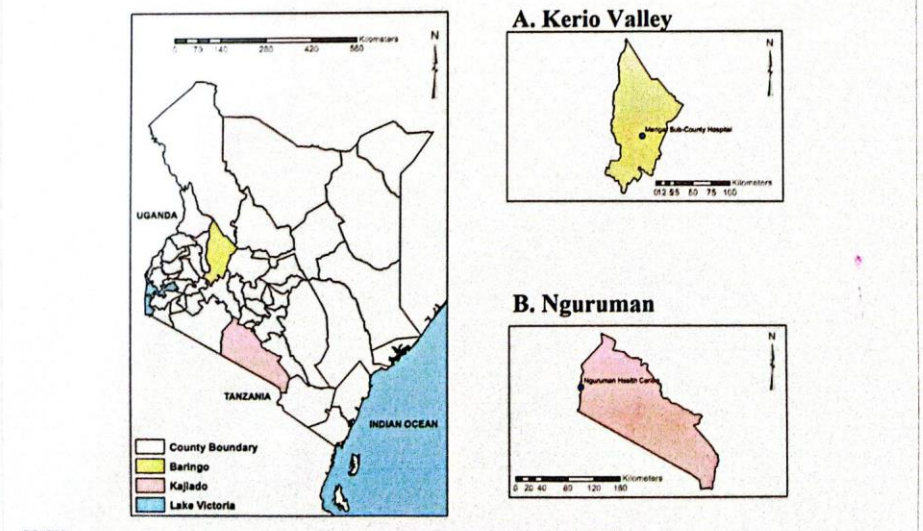

FIGURE 1
Kenyan map displaying the locations of the two study sites. **(A)** Kerio Valley and **(B)** Nguruman. The maps were created with ArcGIS Desktop Version 10.2.2 (Advanced License)

future outbreaks (17, 18). Based on the information given during sampling, the samples tested from Kerio Valley in Baringo County were from participants who were not vaccinated. However, records of vaccination status were based on self-reporting which could not be verified, as no vaccination cards were provided during the sample collection. However, following the 1992/93 YFV outbreak in Rift Valley, routine vaccination was instituted in Baringo County. Most of the participants may not remember being vaccinated.

2.3 Sample collection

An estimated 5ml of venous blood sample was obtained during a cross-sectional hospital-based survey from febrile consenting participants visiting the selected health facilities during the period between July 2020 and May 2023. Blood was separated and serum kept in a liquid nitrogen shipper at the site and transferred to the International Centre of Insect Physiology and Ecology (ICIPE) for further laboratory analysis. Both males and females aged between 5 and 85 years old presenting with a malaria-like fever illness were recruited into the study. A structural questionnaire was administered whereby demographic data on; age, occupation, gender, status of yellow fever vaccination, residence places and any relevant history of travel by participants was obtained.

2.4 Viruses and cell lines

Although the focus was on yellow fever and dengue-2 viruses, testing for neutralizing antibodies for the most common *Flaviviruses*; dengue, yellow fever, Zika, and West Nile viruses, was performed by PRNT to rule out cross reactivity. Dengue-2 was chosen because it is the most common dengue serotype in Kenya (12, 14, 15). The neutralization was done using live preserved viral isolates (DENV-2 008/01/2012, YFV YFXSMB, ZIKV MR766, and WNV AMH005348) obtained from KEMRI's Viral Hemorrhagic Laboratory (VHF). To conduct virus titration and neutralization testing, the viruses were propagated in the C6/36 (Aedes albopictus mosquito) cell lines and inoculated on Vero cell lines (CCL81, and E6).

The inoculated viruses were harvested and quantified using plaque assay as described by (39). Briefly, ten-fold serial dilutions of the viral stocks were prepared. 2.0 ml microcentrifuge tubes (Eppendorf, USA) were labelled as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and Negative. 900µl of maintenance media (Minimum Essential Media Eagles, Sigma, enhanced with 2% heat-inactivated fetal bovine serum (FBS), 2% penicillin/amphotericin B, and 2% L-glutamine (Sigma-Aldrich, St. Louis, MO) was added to each tube. The viruses were serially diluted 10-fold by transferring 100 µl of each virus to the appropriate tube labelled 10^{-1}, vortexing, and transferring 100µl to the subsequent tube, down to 10^{-6} with the last 100µl being discarded. Subsequently, Vero cells (E6) were seeded into a 12-well plate (Corning, USA) and incubated for 24 hours in a 5% CO2 set at 37°C to allow the formation of a confluent monolayer. Next, the plate was labelled properly, and 100µl of the diluted virus (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}) was dispensed into the specified wells, with each pipette tip being changed. A negative control was included in the test. For one hour, the plate was incubated in 5% CO_2 at 37°C, with 15-minute intervals of rocking to facilitate virus adsorption. After adsorption, the cells were overlaid with 1ml methylcellulose (2.5%) (Sigma) mixed with 2X Minimum Essential Medium (Sigma). The well plates were placed in a 5% CO_2 set at 37°C. Following a period of 6 to 14 days, depending on the virus under investigation, the plates were fixed in formalin and stained using crystal violet (diluted in 100% ethanol) for visualization of plaques. The plaques formed were manually counted and calculated to quantify the virus using the formula below (40).

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

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

Viral titer was expressed as plaque-forming units (pfu) per ml and an appropriate virus dilution factor (containing 20-70 plaques) (39) was used to calculate the amount of virus needed for dilution of the virus to be used for PRNT. The calculated viral titers for the viruses used are shown in Table 1.

2.5 Plaque reduction neutralization test

Serum samples were removed from the −80°C freezer, thawed, aliquoted in 30µl volumes, and heat-inactivated for 30 minutes at 56°C. Using the Plaque Reduction Neutralization Test ($PRNT_{90}$), the samples were examined for neutralizing antibodies to the DENV-2, YFV, WNV, and ZIKV viruses in two-fold serum dilutions ranging from 1:10 to 1:5160. The viruses were amplified in cell culture, tittered by plaque assay, and working stock diluted to 20 – 70 plaques per 50µl. Beginning with a seropositivity threshold of 1:10 ten-fold dilutions the serum was prepared using the maintenance media (Minimum Essential Media Eagles, Sigma, enhanced with 2% penicillin/amphotericin B, 2% heat-inactivated fetal bovine serum (FBS), and 2% L-glutamine (Sigma-Aldrich, St. Louis, MO). The diluted serum sample was mixed with 30µl of the known constant concentration of the diluted virus in microcentrifuge tubes followed by a 1-hour incubation at 37°C. The virus-antibody mixture was added to a 24-well plate that contained a confluent monolayer of VERO E6 cell lines. The plates were then incubated for one hour at 37°C in an incubator with 5% CO2 to allow the virus to adsorb. After 1 hour, each well was maintained with 1ml methylcellulose (2.5%) (Sigma) overlay mixed with 2X Minimum Essential Medium (Sigma). After 6 to 14 days, subject to the virus being examined, the plates were fixed with formalin, stained with crystal violet (diluted in absolute ethanol) and plaques counted manually (2, 34, 41). Plaque reduction neutralization test (PRNT) end-point titers (seropositivity threshold) are expressed as the reciprocal of the last serum dilution showing the desired per cent reduction in plaque counts (42). In our study, we reported the endpoint titer as the reciprocal of the highest serum dilution that resulted in ≥ 90% reduction in plaque counts ($PRNT_{90}$) (43). Thus, $PRNT_{90}$ was used to calculate the highest serum dilution (1:10 to 1:5160) required to reduce DENV-2, YFV, WNV and ZIKV plaque formation by 90% in Vero cells (44). In cases where two or more viruses neutralized the same

TABLE 1 Plaque assay results showing the calculated titers of DENV-2, YFV, ZIKV and WNV.

Virus	Passage Number	Number of plaques	Dilution factor	The volume of diluted virus added (µl)	Titer (pfu/ml)
DENV-2 (008/01/2012)	1	48	10^{-2}	100	$4.8X10^{4}$
YFV(YFXSMB)	2	82	10^{-3}	100	$8.2X10^{5}$
ZIKV(MR766)	3	38	10^{-2}	100	$3.8X10^{4}$
WNV(AMH005348)	2	53	10^{-2}	100	$5.3X10^{4}$

YFV, Yellow fever virus; WNV, West Nile virus, DENV-2, Dengue-2 virus; ZIKV, Zika Virus.

sera sample, based on the WHO guidelines and criteria, the virus with an antibody titer of four-fold or higher than the other flaviviruses tested was considered viral-specific and hence positive (42, 45–47).

2.6 Statistical analysis

All the data collected was stored in password-protected folders and analyzed using R software version 4.3.2 (48) and Microsoft Excel. PRNT endpoint titer was computed as the reciprocal of the last serum dilution demonstrating the intended per cent reduction in plaque counts. Thus, the endpoint titer was reported as the reciprocal of the highest serum dilution that produced a reduction in plaque counts of at least 90% ($PRNT_{90}$) (42, 43). The participants were first characterized based on site and demographic characteristics. Descriptive statistics were performed and the prevalence was compared by site and demographic characteristics i.e., gender, age and occupation. The heterogeneity of the seropositive proportions was evaluated and proportions were compared using the Chi-Square test. A multinomial logistic regression model was used to predict risks for each of the most prevalent viruses with covariates in association with age, gender, and occupation data. The odds ratio (OR) generated was used to assess the association between the risk factors and the outcomes. A p-value of 0.05 or less was considered significant at a 95% confidence level.

3 Results

3.1 Demographic characteristics of the study participants

The study participants' demographic characteristics for both the individual and combined study sites are displayed in Table 2. This study population comprised a total of 480 participants, 194 (40.4%) from Nguruman and 286 (59.6%) from Kerio Valley. 60.21% (289/480) were females and 39.79% (191/480) were males. The sampled participants' ages ranged from 5 to 85 years with a mean and median age of 27 years and 24 years respectively. About 37.16% of the participants were farmers, 30.48% were students, 5.64 were businesspeople. 16.08% were housewives, while 1.67% engaged in other economic activities and 8.98% had no indicated occupation. Participants were structured into four age groups; Below 25 years, 26-45, 46-65, and 66 and above. Most of the participants (56.25%) were below 25 years which comprised mostly school-going children. All the participants indicated that they were not vaccinated against the yellow fever virus during the survey.

3.2 Prevalence of neutralizing antibodies against Yellow fever, Dengue-2, and related Flaviviruses in Nguruman and Kerio Valley

Overall, 54.5% (262/480; 95% CI, 50.1–59.0%) of the samples had neutralizing antibodies to at least one of the four flaviviruses screened with higher proportions being detected in in Kerio Valley (64.34%, 184/286) than in Nguruman (40.2%, 78/194) (P<0.0001). From viral neutralization, the seroprevalence of the neutralizing antibodies categorized according to study sites showed that overall, yellow fever virus (32.5%) had the highest prevalence of neutralizing antibodies followed by West Nile (16.25%), Zika (5.2%), and Dengue- 2 (1%) viruses (Table 3). In addition, the seroprevalence of the neutralizing antibodies against flaviviruses varied across the sites. Kerio Valley had a significantly higher YFV seropositivity (51%) than Nguruman (6%) (P<0.0001), while Dengue-2 was observed only in Nguruman with a low seropositivity of 2%. Whereas the rates of ZIKV were comparable, at 5% (13/286) in Kerio Valley and 6% (12/194) in Nguruman, WNV exposure rate was significantly greater in Nguruman (27%, 52/194) than in Kerio Valley (9%, 26/286), (P<0.0001) (Table 3; Figure 2).

Figure 3 presents the seroprevalence of neutralizing antibodies for both study sites based on gender. The findings indicate that in Kerio Valley, seropositivity was significantly higher in females (118/286; 41.26%) than in males (66/286; 23.08%) (P<0001). In Nguruman, however, males' seropositivity rates were higher (47/194; 24.23%; P=0.049).

Seroprevalence of neutralizing antibodies against the four flaviviruses also varied by age group (Figure 4). In Kerio Valley, the prevalence of YFV-neutralizing antibodies was highest in the below 25 years' age group and lowest in the 66+ years age group, a similar pattern was observed for WNV and ZIKV neutralizing antibodies, with the highest prevalence among the below 25 years' group and the lowest in the age group 66+ years. In Nguruman, age was significantly

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TABLE 2 Demographic characteristics of the study population from Nguruman in Kajiado and Kerio Valley in Baringo Counties.

CHARACTERISTIC	NGURUMAN		KERIO VALLEY		COMBINED	
	N	%	N	%	N	%
ALL	194	100	286	100	480	100
SEX						
Male	99	51.03	92	32.17	191	39.79
Female	95	48.97	194	67.83	289	60.21
AGE GROUP (YEARS)						
25 and Below	102	52.6	168	58.74	270	56.25
26 - 45	75	38.7	69	24.12	144	30
46 - 66	16	8.2	37	12.94	53	11.04
66 and above	1	0.5	12	4.2	13	2.71
OCCUPATION						
Farmer	100	51.55	78	27.27	178	37.16
Student	38	19.59	108	37.76	146	30.48
Businessman	4	2.06	11	3.85	15	3.13
Business lady	4	2.06	9	3.15	12	2.51
Housewife	16	8.25	61	21.33	77	16.08
Didn't indicate	5	2.58	3	1.05	8	1.67
Non	27	13.92	16	5.59	43	8.98

associated with WNV seropositivity (P= 0.02) being highest among the age group 26 - 45 years and lowest in the age group 66+ years while DENV-2 neutralizing antibodies' prevalence was highest in the age group 25 and below and lowest in the age group 26 - 45 years.

3.3 Demographic predictors for seroprevalence of the most prevalent Flaviviruses in Kerio Valley and Nguruman

Table 4 shows results estimated by the Multinomial Logistic Regression model. The findings show that in Kerio Valley, men were approximately two times more likely to have been exposed to YFV than females (OR =1.31, 95% CI = 0.26-6.50). There were no significant differences between yellow fever virus prevalence and participants' age and occupation in Kerio Valley. Males were twice more likely to be seropositive than females for WNV in Kerio Valley (OR= 2.19, 95% CI=1.14-4.19). Likewise, WNV seroprevalence was about three times more in the age group 26-45 years than below 25 years (OR = 2.83, 95% CI=1.13-7.12, P value = 0.027). The occupation did not affect WNV seroprevalence. In Nguruman, the likelihood of WNV exposure was about 2.49 times higher in males compared to females (OR =2.49, 95% CI = 1.40-4.42, P=0.002), and a two-fold higher likelihood of WNV in the age group 26 - 45 years relative to under 25 years (OR=2.27, 95% CI = 1.06-4.86, P value= 0.035). There was no observed effect of occupation on WNV exposure in this site.

TABLE 3 Prevalence of neutralizing antibodies against yellow fever, dengue-2, and related flaviviruses in Nguruman and Kerio Valley.

Virus	Nguruman n (%)	Kerio Valley n (%)	Combined n (%)
YFV	11 (6)	145 (51)[a]	156 (32.5)
WNV	52 (27)[b]	26 (9)	78 (16.25)
ZIKV	12 (6)	13 (5)	25 (5.2)
DENV-2	3 (2)	0 (0)	3 (1)

YFV, Yellow fever virus; WNV, West Nile virus, DENV-2, Dengue-2 virus; ZIKV, Zika Virus.
[a]Significant difference between the sites ($p<0.0001$) by the Chi-square test.
[b]Significant difference between the sites ($p<0.0001$) by the Chi-square test.

4 Discussion

Globally, arboviruses such as yellow fever (YFV), dengue (DENV), West Nile (WNV) and Zika (ZIKV) have become a major public health threat with a significant socioeconomic burden (13, 15, 49). In sub-Saharan Africa, these infections mostly go undetected or misdiagnosed in high-risk remote endemic areas that are hard to reach due to inaccessible health facilities, infrastructure or personnel (7). This study analyzed the seroprevalence of yellow fever, dengue-2 virus, and related *Flaviviruses* (West Nile virus and Zika virus) circulating among the human population in Nguruman and Kerio Valley, Kenya using Plaque reduction neutralization test (PRNT). The plaque reduction neutralization test (PRNT) is considered the gold standard for identifying serological specificity among viruses and differentiation of Flavivirus infections (42, 43, 50, 51). We employed a more stringent $PRNT_{90}$ which has a higher specificity and can reduce the background serum cross-reactivities across flaviviruses, hence it is very beneficial for epidemiological and diagnostic studies in dengue and yellow fever endemic areas (42, 51, 52). Thus, the method ruled out any cross-reactivities among the flaviviruses tested. Of the 480 samples that were analyzed, at least one of the four viruses was detected in 54.5% (262/480; 95% confidence interval [CI] 50.1–59.0%). The rates of exposure to these infections for every virus and site were found to be varied. The detected circulating viruses; YFV, DENV-2, WNV, and ZIKV were varied in proportion in both Nguruman and Kerio Valley. Therefore,

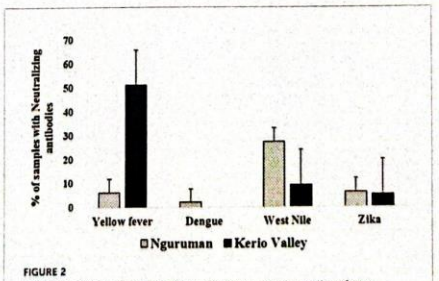

FIGURE 2
Seroprevalence of neutralizing antibodies against yellow fever, dengue-2, and related flaviviruses categorized by study sites. The number of samples tested was n=286 in Kerio Valley and n=194 in Nguruman, the error bars indicate a 95% confidence interval.

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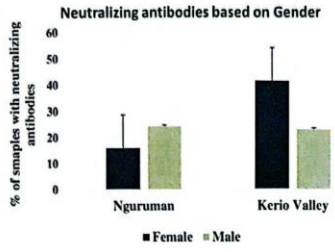

FIGURE 3
Proportion of samples with flavivirus neutralizing antibodies based on gender, for both Nguruman and Kerio Valley, the error bars indicate a 95% confidence interval.

the observed variation was associated with the varying exposure risk to these infections across sampled regions because of the wide vector distribution, diverse climatic and geographical conditions (2, 53).

Moreover, arboviral diseases are known to have wide non-specific clinical presentations and cross-reactivity (54, 55). These variations could also play a role in experiencing challenges in deciding an encountered differential diagnosis due to shared similar febrile clinical presentation illnesses by related viruses or cross-reactivity of these arboviruses in laboratory diagnosis (7, 56, 57). As a result, it is important to raise awareness among doctors and the general public about arbovirus infections as potential causes of febrile illnesses. Additionally, providing diagnostic tools at health facilities could aid in the identification and distinction of arboviral infections from other febrile conditions (54). Nevertheless, Kerio Valley is one of the regions in the country that has poor access to health facilities, infrastructure and personnel following cattle rustling and insecurity (58). Therefore, the unavailability of arboviral diagnostic capacity within the available health facilities could also limit tracking or conducting surveillance of these flaviviruses. Thus, the data from the study will be key in planning surveillance and diagnostic capabilities.

In this study, YFV-neutralizing antibodies with significantly higher rates were detected in Kerio Valley (145/286, 51%) compared to Nguruman ($P<0.0001$). The observed high rates of arbovirus exposure may be attributed to the prior mass yellow fever vaccination conducted following the YFV outbreak that occurred in Baringo in 1992/93 and subsequent routine YF immunization instituted for children at 9 months (17, 18). Regardless of the level of disease protection that could be maintained after three decades of routine immunization, Yellow fever infections could still occur if sufficient herd immunity is not attained. In addition, mosquito vectors associated with yellow fever transmission; *Aedes africanus* and *Aedes keniensis* are still circulating in Baringo County (18, 59, 60) and could still transmit this virus. This study further suggests the

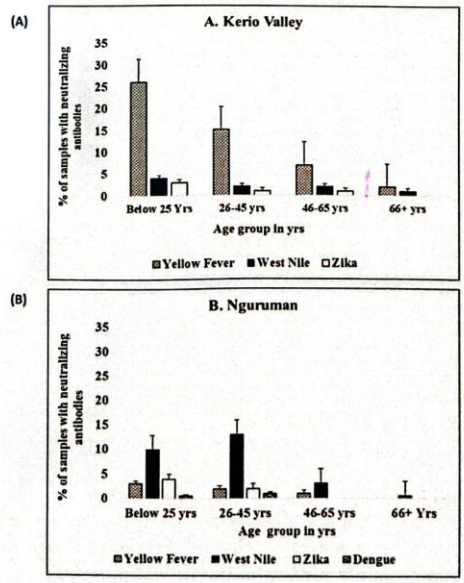

FIGURE 4
Proportion of samples with flavivirus neutralizing antibodies by age group in **(A)** Kerio Valley, n=286 and **(B)** Nguruman, n =194, the error bars indicate a 95% confidence interval.

TABLE 4 A comparative analysis of the two most prevalent flaviviruses (YFV and WNV) in Nguruman (Kajiado County) and Kerio Valley (Baringo County) by age, gender and occupation using Multinomial Logistic regression model.

Variable	Kerio Valley				Nguruman			
	Yellow fever (YFV)		West Nile (WNV)		Yellow fever (YFV)		West Nile (WNV)	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
SEX								
Female	Reference		Reference		Reference		Reference	
Male	1.31(0.26-6.50)	0.7	2.19 (1.14-4.19)	0.018*	0.78 (0.01-46.2)	>0.9	2.49 (1.40-4.42)	0.002*
AGE								
25yrs & below	Reference		Reference		Reference		Reference	
26-45yrs	0.74 (0.13-4.16)	0.7	2.83 (1.13-7.12)	0.027*	0.81 (0.27-2.43)	0.7	2.27(1.06-4.86)	0.035*
46-65yrs	0.32 (0.03-3.64)	0.4	1.95 (0.66-5.72)	0.2	0.30 (0.03-2.73)	0.3	1.72 (0.66-4.45)	0.3
66yrs & above	0.00 (0.00-0.00)	<0.001	2.60 (0.45-15.0)	0.3	0.00 (0.00- 0.00)	>0.9	2.28 (0.41-12.8)	0.3
OCCUPATION								
Business lady	Reference		Reference		Reference		Reference	
Businessman	0.32 (0.01-10.3)	0.5	0.22 (0.03-1.88)	0.2	0.42 (0.02-9.33)	0.6	0.24 (0.04-1.62)	0.14
Farmer	0.22 (0.01-3.93)	0.3	0.36 (0.06-2.09)	0.3	0.51 (0.05-5.25)	0.6	0.45 (0.10-1.94)	0.3
Housewife	0.98 (0.06-14.8)	>0.9	0.48 (0.08-2.93)	0.4	1.56 (0.15-16.3)	0.7	0.69 (0.15-3.15)	0.6
Didn't indicate	0.00 (0.00-0.00)	<0.001	0.15 (0.01-3.15)	0.2	0.00 (0.00-0.00)	<0.001	0.19 (0.01-2.78)	0.2
Student	0.15 (0.01-2.11)	0.2	0.35 (0.06-1.95)	0.2	0.39 (0.03-4.09)	0.4	0.43 (0.10-1.85)	0.3

*Indicates statistically significant value.

existence of a low-level active circulation of YFV in Nguruman with 6% neutralizing antibodies being detected. However, up to date, no documented outbreak of YFV in Nguruman has been reported. None of the participants in Nguruman were vaccinated against the yellow fever virus. Furthermore, economic activities like wild animals, forested conservancies and subsistence farming with intensive irrigation in Nguruman provide mosquito breeding grounds. The herders' frequent travels in search of pasture and water in these forested conservancies increase and provide human-sylvatic mosquito contact hence this human activity of encroaching on forest ecosystems offers YFV a reservoir and a means of transmission (53). Additionally, human migration into urban areas, population growth, climate change, and changes in land use (such as deforestation) are all possible causes that could have increased *Aedes* mosquito numbers and aided transmission of the Yellow fever virus (2). Additionally, the majority of the flaviviruses are dependent on suitable environmental condition that favors the survival and distribution of their vectors and thus are likely to re-emerge in new locations with favorable conditions (61–63). Thus, our study shows an increased risk of continued sylvatic transmission coupled with the suitable ecological factors that could favor Flavivirus transmission, even in previously naïve regions like Nguruman. Thus, monitoring the risk of transmission of these flaviviruses should provide information for surveillance of flaviviruses in the future and preparedness for outbreaks in the area.

DENV-2 seropositivity was detected only in Nguruman. Dengue outbreak was first reported along the Kenyan coast in 1982 (12). Ever since, several positive cases have been detected suggesting a likelihood of the regional spread of the virus (49). In 2011, the dengue virus re-emerged, leading to outbreaks in the coastal and northern regions of Kenya (15, 64). In addition, low endemicity of Dengue infections has been reported confirming the occurrence of DENV in areas outside the initial geographical outbreak boundaries (15, 49, 56, 65). The climate change and a dryland ecosystem in Nguruman could favor a high abundance of *Aedes aegypti* mosquito vectors that could be contributing to the transmission of DENV. Other studies have shown that the primary dengue vector, *Aedes aegypti*, is widely distributed throughout the country confirming the underlying risk of Dengue outbreaks (38, 60, 66, 67). These findings may indicate the continued spread of dengue from the Kenyan coast where it has been more common, thus, there is a need for continuous Dengue serosurvey to avert possible outbreaks.

West Nile virus (WNV) neutralizing antibodies were detected in both study sites with high frequency (40.2%) being detected in Nguruman. The observed frequency was found to be higher than

those previously detected in Turkana (10.2%) (2). The existence of a conservancy park, lakes and rivers in both Nguruman and Kerio Valley could be associated with high-frequency detected WNV following the provision of good breeding grounds for *Culex* mosquito species and the existence of diverse bird species, which are also important in WNV transmission cycles (2, 28, 68). The rapid adaptation of WNV to infect local mosquito vectors is responsible for the virus's wider epidemiological dissemination (62).

Although there has never been a reported or confirmed outbreak of Zika in Kenya, our findings suggest the circulation of Zika in Nguruman and Kerio Valley counties. The prevalence of Zika was detected in the studied regions with almost the same proportions in both Kerio Valley (5%) and Nguruman (6%). Studies on human exposure to ZIKV in Kenya are low (2, 32, 33). The detected low proportion of Zika exposures could be attributed to inadequate clinical and laboratory facilities rendering the disease unnoticed/undetected. Both Nguruman and Kerio Valley have an abundance of mosquito vectors whereby human interaction with sylvatic mosquitoes with the transmission maintained from the frequent movement of residents in search of pasture, firewood or water. Furthermore, there have been proposals to attribute the rise of ZIKV infections in people to ecological reasons, which are a result of increased mosquito transmission by *Aedes species* (53). This ecosystem could be targeted for arboviral surveillance to prevent viral transmission that could result in outbreaks.

The seropositivity also varied by gender and age groups in both Kerio Valley and Nguruman. Females in Kerio Valley had significantly higher neutralizing antibodies than males. This reflects the enrollment rates in the study also concentrated in the same gender where more females 60.21% (n=289) than males 39.79% (n=191) were enrolled. The high enrollment of women may be influenced by the tendency of women tend to exhibit hospital-seeking behaviors as compared to men. Men being away from homes mostly grazing livestock and farming may influence their low level of enrollment. Men were also more likely to be infected with YFV and WNV than females. This could be explained by the activities males perform such as farming, looking for pasture and taking care of livestock whereby these kinds of outside activities predispose them to infectious mosquito bites. This could account for the glaring difference between the exposure rates of men and women within study areas.

Most of the participants (56.25%) were below the age of 25 years which mainly comprised school-going children. In Kerio Valley, seropositivity declined with age. Those below 25 years' age group had higher neutralizing antibodies to YFV, with a similar pattern observed for WNV and ZIKV. In Nguruman, neutralizing antibodies against WNV were highest in the 26-45 years' group with the odds of exposure being more in the same age group which may indicate low-level ongoing and persistent transmission of this virus in the study areas. Active virus transmission is supported by evidence of virus isolation from mosquitoes of Culex spp. in this ecosystem (38, 68).

This study had limitations. For instance, our study was focused only on a single serotype (DEN-2) indicating the need for an additional study on DENV genetic diversity to ascertain the precise prevalence of dengue in the studied region and its serotype distribution.

5 Conclusion

The findings from the study suggest that there is circulation of the *Flaviviruses*; YFV, WNV and Zika viruses of great public health importance in both Nguruman and Kerio Valley that could be contributing to under-recognized clinical disease. This study confirms the need to administer vaccines against these viruses to a level (80%) that could achieve herd immunity besides the implementation of vector control strategies to mitigate the potential risk of an outbreak.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The study was approved by the Scientific Ethics and Review Unit (SERU) of the Kenya Medical Research Institute (KEMRI) (protocol number KEMRI/SERU/CVR/013/4664). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

MK: Conceptualization, Formal analysis, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing, Investigation, Data curation. EC: Conceptualization, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing, Formal analysis. SM: Supervision, Validation, Writing – original draft, Writing – review & editing. DT: Conceptualization, Funding acquisition, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. RS: Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing, Funding acquisition, Resources, Project administration.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fviro.2024.1459021/full#supplementary-material

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