VIRAL PROFILE OF CULEX SPECIES COLLECTED FROM PARTS OF ACCRA AND NAVRONGO

BY

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DEDICATION

This is dedicated to myself, my mum, Ms Henrietta Tsotso Lartey, my family, and everyone else who helped make it a success. Your guidance, support, and love kept me going, and I sincerely appreciate you all.

Thank you, and God bless us all.

DECLARATION

I, Kevin Nii Yartey Yartey, hereby declare that this work is a product of the research carried out at the NAMRU-EURAFCENT lab of the Noguchi Memorial Institute for Medical Research (NMIMR), under the supervision of Prof. Samuel K. Dadzie (NMIMR) and Dr. Mildred Adusei-Poku (Department of Medical Microbiology, University of Ghana Medical School) and that all references cited have been duly acknowledged.

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List of Abbreviations

ACDC – Africa Centre for Disease Control

CDC – Centre for Disease Control

cDNA – Complementary DNA

CHIKV – Chikungunya virus

Cx. – Culex

DENV – Dengue virus

DNA – Deoxyribonucleic Acid

dNTP - Deoxynucleotide Triphosphates

ELISA – Enzyme-Linked Immunosorbent Assay

HIV – Human Immunodeficiency Virus

IFA – Immunofluorescence Assay

IgG – Immunoglobulin G

IgM – Immunoglobulin M

ISV – Insect-specific viruses

JEV – Japanese Encephalitis Virus

LAMP – Loop-mediated isothermal amplification

MDA – Multiple Displacement Amplification

mNGS – Metagenomic next-generation sequencing

NCBI – National Centre for Biotechnology Information

NGS – Next Generation Sequencing

NHRC – Navrongo Health and Medical Research Centre

PAHO – Pan American Health Organisation

PCR – Polymerase Chain Reaction

qPCR – Quantitative Polymerase Chain Reaction

qRT-PCR – Quantitative Reverse Transcriptase Polymerase Chain Reaction

RNA – Ribonucleic Acid

RT-PCR – Reverse Transcriptase Polymerase Chain Reaction

RVFV – Rift Valley Fever virus

SLEV – St. Louis Encephalitis virus

SSA – sub-Saharan Africa

ssRNA – Single-Stranded RNA

TAE – Tris-Acetate Ethylenediaminetetraacetic Acid

WHO – World Health Organisation

WNV – West Nile Virus

YFV – Yellow fever virus

ZIKV – Zika virus

ABSTRACT

Background: *Culex* mosquitoes are prevalent in Ghana and play an under-recognised role in arbovirus transmission. However, a proper characterisation of the specific viral profiles carried by these mosquitoes in Ghana is lacking. Rapid urbanisation in Ghana, particularly in Accra, coupled with contrasting ecological conditions in peri-urban Navrongo, creates favourable environments for Culex proliferation and potential arbovirus circulation. This study profiled viral pathogens and broader virome diversity in Culex populations from these two ecologically distinct settings.

Methods: A total of 2,886 *Culex* mosquitoes were collected from parts of Accra and Navrongo and organised into 217 pools for analysis. Conventional PCR assays targeting pan-flaviviruses and pan-alphaviruses were performed on all 217 pools, followed by metagenomic next-generation sequencing (mNGS) on 20 pools. Ten (10) out of the 20 pools yielded reads for downstream analysis. Host-deleted reads were taxonomically classified using Kraken 2, assembled with SPAdes, and viral contigs were annotated with BLASTn. Phylogenetic analysis of major viral families was conducted using maximum likelihood methods.

Results:

Pan-flavivirus and pan-alphavirus PCR assays yielded no detectable amplification in all *Culex* pools, suggesting the absence of active infections with classical human-pathogenic arboviruses. Conversely, mNGS revealed a diverse virome comprising 18 viral families. Phylogenetic analysis showed that Ghanaian avian leukosis and myelocytomatosis virus sequences clustered tightly with Indian, Chinese, and American strains, indicating possible historical or ongoing cross-continental viral exchange.

Conclusion:

This study provides a virome profile of *Culex* mosquitoes in Ghana, revealing viral diversity despite the absence of major human-pathogenic arboviruses. The findings underscore the ecological complexity of *Culex* viromes, highlight potential transboundary viral introductions, and demonstrate the value of mNGS for arbovirus surveillance.

CHAPTER ONE

1.0. INTRODUCTION

1.1 Background

Arbovirus, short for arthropod-borne viruses, represent a diverse group of mostly RNA viruses transmitted primarily through blood-feeding arthropods such as mosquitoes, ticks, biting midges, and sandflies. These viruses show similar ecological epidemiology (Calisher, 1994; Casals, 1972; Davis et al., 2008) and are categorised into different families, each distinguished by structure, genome, and replication mode. These are the alphaviruses (genus Alphavirus, one of two genera in the family Togaviridae) (Chen et al., 2017), flaviviruses (genus Flavivirus, one of three genera in the family Flaviviridae) (Simmonds, et al., 2017), bunyaviruses, nairoviruses, and phleboviruses (three of five genera in the family Bunyaviridae) (Horne & Vanlandingham, 2014; Hughes et al., 2020; Kuhn et al., 2016), orbiviruses (one of nine genera in the family Reoviridae) (Belaganahalli et al., 2015), vesiculoviruses (one of six genera in the family Rhabdoviridae) (Temmam et al., 2016; P. J. Walker et al., 2018), and thogotoviruses (one of four genera in the family Orthomyxoviridae) (Temmam et al., 2016). These families are characterised by single-stranded or double-stranded RNA genomes, enveloped or non-enveloped structures, and different transmission cycles between arthropod vectors and vertebrate hosts. The viral transmission cycle typically involves a hematophagous arthropod vector that acquires the virus by feeding on an infected host and transmits it to a new host during later feedings (Attoui et al., 2020).

Arthropod-borne viruses were a significant threat in the 19th and 20th centuries. However, they are now overshadowed by newer viruses such as hepatitis and HIV, which are known for causing chronic conditions (Rosenberg et al., 2013). This makes arboviruses a secondary concern in global health, particularly in developed nations, where the concept of "emerging viruses" is considered

outdated (Charrel, 2021). Ticks, mosquitoes, and sandflies effectively transmit arboviruses; nevertheless, ticks and mosquitoes are the primary vectors, with hard ticks harbouring the highest viral diversity (Ergunay, 2014; Viglietta et al., 2021). Mosquitoes are notorious for their ability to transmit viral pathogens such as the Zika virus, West Nile virus, Dengue virus, Yellow Fever virus, Chikungunya virus, and Rift Valley Fever virus (Chen et al., 2024; Makhanthisa et al., 2024).

Arboviruses pose significant public health challenges due to their ability to cause outbreaks of severe diseases with high morbidity and mortality rates. These viruses cause notable emerging and re-emerging infectious diseases, such as dengue, chikungunya, West Nile, and Zika. Arboviral diseases have global implications, affecting millions of people annually (Ali, 2023; Côrtes et al., 2023a; Majumdar & Jana, 2022; PAHO, 2023). Infections can manifest as febrile illnesses, haemorrhagic fevers, encephalitis, arthritis, and other severe neurological conditions (Beckham & Tyler, 2015a; Sigfrid et al., 2018) The burden of arboviral infections is worsened by climate change, urbanisation and increased human mobility, leading to the spread of vectors and the viruses they carry (Chikezie et al., 2024).

Agboli et al (2021) report that since 2017, West Africa has reported 27,000 arboviral outbreaks, making it a highly vulnerable region for future outbreaks. The 2022 WHO report on arboviral surveillance indicates that West Nile and Rift Valley Fever Viruses are in circulation in Africa. Due to globalisation and the extended incubation periods of these viruses, they are well-positioned as ideal candidates for the global spread. According to the WHO report on arboviral surveillance, West Nile Virus and Rift Valley Fever Virus are notably prevalent worldwide (WHO, 2022). Countries with these arboviruses are now connected to those with little or no virus through travel and other trade activities. This link allows the virus to spread to new geographic regions when infected mosquitoes feed on uninfected individuals. The virus can then be transmitted from

humans to mosquitoes and back again, creating a vicious transmission cycle (Chiuya et al., 2021). *Culex*-borne viruses such as West Nile Virus (WNV) and Japanese Encephalitis Virus (JEV) are associated with significant morbidity and mortality, particularly among vulnerable populations, including the elderly, young children, and immunocompromised individuals (Krambrich et al., 2024; Oliveira et al., 2020). These viruses can cause severe neurological diseases, leading to long-term disability and economic burdens on healthcare systems.

Several notable outbreaks of *Culex*-borne viruses have occurred worldwide (Gould et al., 2025; Hoxha et al., 2025; Mussetto et al., 2025). The WNV was first discovered in Africa in 1937, after its 1999 introduction to the United States, there have been widespread outbreaks across North America, with thousands of cases and hundreds of deaths (Garmendia et al., 2001; Ronca et al., 2021). Similarly, the Japanese encephalitis virus continues to cause recurrent outbreaks in 24 countries in Southeast Asia and the Western Pacific, placing about three billion people at risk of infection (Simon & Kruse, 2019) . In Asia, vaccination programs are being implemented to reduce the disease burden (WHO, 2024b)

1.2. Problem Statement

Mosquito-borne diseases, particularly arboviral infections, pose a significant global public health challenge. Among mosquito vectors, *Culex* species play a central role in transmitting arboviruses such as West Nile virus (WNV), Japanese Encephalitis virus (JEV), Rift Valley Fever virus (RVFV), and St. Louis Encephalitis virus (SLEV) to humans (Dunphy et al., 2022; Tolsá-García et al., 2023). In West Africa, arboviral diseases are increasingly recognised as a public health concern (Manouana et al., 2024; Wikel et al., 2024), yet little is known about the viral landscape of *Culex* mosquitoes in urban areas such as Accra and Navrongo.

Rapid urbanisation in Ghana has created favourable ecological conditions for *Culex* proliferation, thereby heightening the risk of viral transmission. While *Aedes* mosquitoes have been the subject of extensive research and surveillance for their role in spreading Dengue and Zika, *Culex* mosquitoes, on the other hand, are typically subjected to distribution studies in Ghanaian societies (Owusu-Asenso et al., 2025). This knowledge gap hinders the ability to anticipate and respond to potential outbreaks associated with *Culex*-borne viruses.

Accra, one of Africa's most rapidly urbanising cities, and Navrongo, a town with contrasting ecological characteristics, represent distinct environments where *Culex* mosquitoes thrive. However, the absence of comprehensive viral profiling data from these locations limits the capacity to assess the epidemiological risks posed by these vectors.

Despite the growing recognition of arboviral threats, national surveillance in Ghana remains primarily focused on malaria and neglected tropical diseases. Arbovirus research is both underfunded and understudied, resulting in limited data on *Culex* populations and the pathogens they harbour. This study, therefore, seeks to fill this critical gap by profiling viral pathogens associated with *Culex* mosquitoes in Accra and Navrongo. Determining the prevalence and genetic diversity of these viruses will generate insights into the public health risks posed by *Culex* populations across urban and semi-urban settings.

1.3. Justification

This research is justified by the increasing public health risks posed by arboviruses transmitted by *Culex* mosquitoes as seen in Cameroon, the Central African Republic and Chad (Poungou et al., 2023). Globally, viruses such as WNV, JEV, and RVFV have caused significant morbidity and mortality (WHO, 2024a). Understanding the vector ecology, behaviour dynamics and epidemiology of Culex-borne viruses would impact the scope of surveillance by providing a

contrasting setting within peri-urban and rural areas. Profiling viral pathogens in these two distinct regions will inform context-specific control strategies and improve preparedness for potential outbreaks.

Furthermore, Ghana's vector control and surveillance programmes have traditionally prioritised malaria, leaving arboviruses understudied and under-detected. This lack of surveillance capacity increases the likelihood that outbreaks of diseases such as West Nile fever or Rift Valley fever will go unnoticed until they escalate. By addressing this deficiency, the present study will provide essential data on transmitted arboviruses, contributing to early detection and prevention.

Additionally, characterising the genetic diversity of viral pathogens will shed light on their evolution and transmission dynamics, offering valuable insights for public health policy and intervention design. Beyond contributing to local disease preparedness, the study will also strengthen the global evidence base on the role of *Culex* mosquitoes in arboviral transmission. Ultimately, the findings will support the integration of *Culex* mosquitoes into national mosquito surveillance frameworks and guide future research on environmental and genetic factors influencing arbovirus transmission in diverse ecological settings.

1.4. Aims or Objectives of Study

This study aimed to conduct a comprehensive viral profiling of *Culex* mosquitoes collected from urban Accra and Navrongo to enhance the understanding of their role in disease transmission.

1.5. Specific Objectives

1. To determine the occurrence of viral pathogens carried by *Culex* spp. in parts of Accra and Navrongo through molecular analysis.
2. To characterise and analyse the genetic diversity of viruses detected in *Culex* mosquitoes through sequencing approaches.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Background

Culex mosquitoes are versatile and adaptable to diverse environments, except extreme northern latitudes such as Antarctica (Biosciences, 2025; Opoku et al., 2007), and they have a broad range of host preferences for blood meals and occasionally cross-feed between avian and mammalian hosts (Sawabe et al., 2010). Given their adaptability and extensive host range, it is crucial to understand the public health implications of *Culex* mosquitoes' behaviour and their role in arbovirus transmission. These characteristics make them ideal vectors for carrying and spreading arboviruses, predominantly zoonotic in nature. This literature review aims to synthesize current knowledge on the ecology, biology, and public health significance of *Culex* species, with a particular emphasis on their feeding habits, arbovirus transmission capacities, theories on arbovirus spread, resistance patterns, implications for vector control, as well as arboviruses, vectors transmitting these viruses, and the global epidemiology of arboviruses with a special focus on Africa and Ghana. *Culex* mosquitoes serve as vectors for several arboviruses, including those responsible for febrile illnesses that often go undiagnosed in regions where malaria and *Aedes* arboviruses are prioritised (Ali et al., 2022). This finding necessitates a deeper understanding of their role in disease transmission and the factors influencing their proliferation. *Culex* mosquitoes are often overlooked during surveillance, despite the increasing incidence of arbovirus transmissions across the region and the potential public health implications calling for a focus on *Culex* spp. for arboviruses in Sub-Saharan Africa. The proposed study areas are significant for *Culex* research, given that Accra is urban with characterised high insecticide resistance, while

Navrongo is rural with ecological settings favourable for vector proliferation (Joannides et al., 2020; Kudom et al., 2015).

Even though *Culex* mosquitoes are efficient vectors for Rift Valley Fever Virus (RVFV) and West Nile Virus (WNV) in West Africa and are circulating in Ghana, surveillance is very limited in Ghana, with one entomological survey in 2009 and a seroprevalence study in 2023 (Agboli et al., 2022; Bangoura et al., 2024; Clark et al., 2018; Johnson et al., 2023; Mencattelli et al., 2022; Tinto et al., 2023; Wang et al., 2009). The contrasting environments of Accra (urban) and Navrongo (peri-urban) give interesting ecological niches for *Culex* mosquitoes, and an interrogation into the virome would provide invaluable insight into zoonotic spillovers, yet no such study has been found. New technologies like metagenomic next-generation sequencing (mNGS) allow for the detection of genetic material in the mosquito and enable identification of both common and rare arboviruses, as well as co-infections and novel viruses that traditional PCR-based methods might miss (Batovska et al., 2022; Coffey et al., 2014; Davis et al., 2008; Souza et al., 2022). In regions where clinical symptoms are non-specific, when standard tests yield inconclusive results, or when resources are limited, mNGS offers a better insight into disease epidemiology (Mendes dos Santos et al., 2022; Yek et al., 2022). Coupled with advanced bioinformatics, mNGS can reveal transmission hotspots and inform targeted interventions by decoding complex viromes.

2.2 Arboviruses: A Global and Regional Public Health Crisis

West Nile Virus (WNV), Rift Valley Fever Virus (RVFV), Dengue Virus (DV), Yellow Fever Virus (YFV), Chikungunya Virus (CV), are responsible for clinical manifestations such as severe to mild febrile illness, neurological and haemorrhagic syndromes, and these viruses are collectively known as Arboviruses. Arthropod-borne virus (Arbovirus) is a label used to describe a large group of viruses, usually pathogenic from diverse families and transmitted primarily by

mosquitoes and ticks, with profound public health impacts (Côrtes et al., 2023b; Paixão et al., 2018a; Salimi et al., 2016; Sukhralia et al., 2019; Young, 2018). Examples of such notable families are Flaviviridae, Togaviridae, Peribunyaviridae, Reoviridae, and Rhabdoviridae.

Clinically, these viruses present varying spectra of illnesses, as mentioned above. These clinical manifestations elucidate the significance that extends beyond acute pathologies, as many arboviruses can cause long-term neurological, ophthalmologic, and cognitive sequelae. With over 250 arboviruses described, at least 80 are pathogenic to humans, making them a formidable public health challenge (Braack et al., 2018; Gan & Leo, 2014; LaBeaud et al., 2011).

2.2.1 Key Culex-Borne Pathogens

The broad family of arboviruses includes Flaviviridae, Togaviridae, Peribunyaviridae, Reoviridae, and Rhabdoviridae, which are mainly RNA viruses and spread by blood-feeding arthropods (Beckham & Tyler, 2015b; Madewell, 2020; McGraw Hill Medical, 2025).

2.2.1.1. Flaviviridae

The flavivirus family includes most of the major pathogens of global and public health significance, such as Yellow Fever Virus (YFV), Dengue Virus (DV), Zika Virus (ZV), West Nile Virus (WNV), Japanese Encephalitis Virus (JEV), and Tick-Borne Encephalitis Virus (TBEV). They are a group of small enveloped, single-stranded enveloped positive-sense RNA genomes ranging from about 9,000 to 13,000 bases (Simmonds et al., 2017; Westaway et al., 2020). Structurally, they have a lipid envelope with a single glycoprotein (E) on their surface, and their RNA genome acts directly as messenger RNA for protein synthesis, and their replication strategy is distinct from related virus families, by manipulating host cell pathways to facilitate their own replication and evade host immune responses (Chen et al., 2017; Neufeldt et al., 2018; Westaway et al., 2020). This family has four main genera

1. Flaviviruses (Orthoflavivirus) include major human pathogens such as yellow fever virus, dengue virus, Zika virus, West Nile virus, Japanese encephalitis virus, and tick-borne encephalitis virus, causing diseases ranging from fever and hemorrhagic syndromes to encephalitis and birth defects like microcephaly (Nelson & Ploss, 2024; Simmonds et al., 2017; Westaway et al., 2020).
2. Hepacivirus includes hepatitis C, which causes liver disease (Hartlage et al., 2016; Thézé et al., 2015).
3. Pestivirus infects livestock and causes fatal mucosal disease, commonly known as bovine viral diarrhoea virus (BVDV) (Government South Australia, 2022; Postel et al., 2021; Sakoda, 2011; Walter Grünberg, 2024)
4. Pegivirus infects mammals with unclear clinical significance, also known as Hepatitis G (ViralZone, 2025; Wu et al., 2020; Yu et al., 2022).

2.2.1.2. Togaviridae

Viruses belonging to this family are small and enveloped with single-stranded, positive-sense RNA genomes of about 10–12 kilobases. Togaviridae used to have two main genera: Alphavirus and Rubivirus. The Alphavirus genus has many mosquito-borne viruses that are important human and animal pathogens, such as chikungunya virus, eastern, western, and Venezuelan equine encephalitis viruses, Sindbis virus, and Mayaro virus (Adouchief et al., 2016; Guzmán-Terán et al., 2020; Hernandez-Valencia et al., 2023; Powers et al., 2015; Rougeron et al., 2015). Mosquitoes typically transmit these viruses to a variety of vertebrate hosts, including birds, rodents, horses, and humans, and can cause diseases ranging from mild fever and rash to severe encephalitis or chronic arthritis (Adouchief et al., 2016; Amaral et al., 2020; Guzmán-Terán et al., 2020; Hernandez-Valencia et al., 2023). However, the Rubivirus genus has only the rubella virus, which

is transmitted between humans via respiratory droplets and is not arthropod borne. This genus has been reassigned to Matonaviridae (Chen et al., 2018; Mankertz et al., 2022).

2.2.1.3. Peribunyaviridae

Viruses in this family have three (3) genomic segments: the S, L, and M, which are negative-sense single-stranded RNA. Genome sizes range from about 10.7–12.5 kilobases (de Melo et al., 2018; de Souza et al., 2024; Hughes et al., 2020; Marklewitz et al., 2013; Rodrigues et al., 2014). The family includes several genera, such as Orthobunyavirus, Herbevirus, Pacuvirus, and Shangavirus, and its members are found worldwide, often kept in cycles involving both vertebrate hosts and arthropod vectors like mosquitoes and biting midges (de Souza et al., 2024; Hughes et al., 2020; Kopp et al., 2019). Some peribunyaviruses are restricted to arthropods, while others can infect humans and animals, typically through the bite of an infected vector. Notable viruses are Oropouche virus, La Crosse Virus, and Schmallenberg virus, causing human and animal infections with clinical outcomes ranging from mild febrile illness to severe neurological or congenital diseases, depending on the specific virus strain (Alissa et al., 2024; Bayrou et al., 2023; Goldman & Hamer, 2024; O'Connor et al., 2024; Zhang et al., 2024). The segmented genome allows for reassortment between viruses, contributing to genetic diversity and the emergence of new strains (Kapuscinski et al., 2021). Peribunyaviruses also encode non-structural proteins that help them evade host immune responses and promote viral replication (Leventhal et al., 2021). There are currently no licensed vaccines or specific antiviral treatments for most peribunyaviruses, making vector control and surveillance critical for prevention (O'Connor et al., 2024; Zhang et al., 2024).

2.2.1.4. Phenuiviridae

Phenuiviridae is a diverse family of viruses within the Bunyavirales order, characterised by segmented, negative-sense RNA genomes that can range from 2 to 8 segments and total 8.1–25.1

kilobases (Sasaya et al., 2023; Sun et al., 2022). Members of this family infect a wide array of hosts including humans, livestock, birds, plants, fungi, and arthropods and a lot of these viruses are typically transmitted by arthropod vectors such as mosquitoes and ticks (Koch et al., 2021; Sasaya et al., 2023; Sun et al., 2022). Notable human and animal pathogens in this family include Rift Valley fever virus, Sandfly Fever Viruses, and Dabie bandavirus, which can cause severe disease outbreaks and have significant impacts on public health, agriculture, and livestock industries (Alkan et al., 2016; Koch et al., 2021; Sasaya et al., 2023; Sun et al., 2022). Some phenuiviruses, like the Rice stripe tenuivirus, are major plant pathogens, highlighting the family's broad ecological and economic relevance (Sasaya et al., 2023). Phenuiviruses encode non-structural proteins (NSs and NSm) that play key roles in antagonising host immune responses, promoting viral replication, and facilitating transmission by vectors (Leventhal et al., 2021). The segmented genome structure allows for genetic reassortment, contributing to the emergence of new strains and complicating control efforts (Kapuscinski et al., 2021). With climate change and global trade, the geographic range of many phenuiviruses and their vectors is expanding, increasing the risk of emerging diseases. Currently, there are limited vaccines or treatments for most phenuiviruses, making surveillance, vector control, and further research essential for managing their impact (Hartman & Myler, 2023)

2.2.1.5. Reoviridae

This family is made up of non-enveloped, double-stranded RNA viruses with segmented genomes, and notable human and animal pathogens within this family are Colorado tick fever virus (CTFV) and bluetongue virus (BTV) (Attoui et al., 2000; National Cancer Institute, 2020; Roy, 1992). CTFV, the type species of the Coltivirus genus, is transmitted to humans primarily through the bite of infected Rocky Mountain wood ticks and is common in northwestern North America; it

causes symptoms like sudden fever, muscle aches, headache, and sometimes rash or gastrointestinal issues, with illness usually resolving in 10–14 days but sometimes leading to prolonged recovery (National Cancer Institute, 2020; Warrell & Warrell, 2012; Yukl & Wong, 2016). Diagnosis of CTFV relies on detecting viral antigen in blood or serological tests, and there is no specific treatment or vaccine; prevention focuses on tick avoidance (Warrell & Warrell, 2012) Bluetongue virus, the prototype of the Orbivirus genus, infects domestic and wild ruminants (especially sheep and cattle) and is transmitted by biting midges; it can cause outbreaks with high morbidity and mortality in livestock, leading to significant economic losses, though many infections are subclinical (Roy, 1992). Reoviruses exhibit great diversity, as advances in sequencing have revealed; this includes a newly identified Coltiviruses related to CTFV in ticks from Australia. This highlights the potential for emerging tick-borne diseases (Harvey et al., 2019). Overall, Reoviridae viruses are significant for their impact on human and animal health, their complex transmission cycles involving arthropod vectors, and their genetic diversity (Harvey et al., 2019; Roy, 1992; Warrell & Warrell, 2012).

2.2.1.6. Asfarviridae

Asfarviridae is a family of large, double-stranded DNA viruses best known for the African swine fever virus (ASFV), which is the only well-characterised member and the causative agent of African swine fever, a highly contagious and often fatal disease in domestic pigs and wild boar (Alonso et al., 2018; Galindo & Alonso, 2017; Gaudreault et al., 2020; Wang et al., 2021). ASFV has a complex structure with an internal core, lipid membranes, and an icosahedral capsid, and its genome ranges from 170 to 194 kilobase pairs (Alonso et al., 2018; Wang et al., 2021). The virus is unique as the only known DNA arbovirus, transmitted both by direct contact and by soft ticks of the genus *Ornithodoros*, which help keep the virus in a sylvatic cycle involving wild pigs in

Africa (Alonso et al., 2018; Galindo & Alonso, 2017; Gaudreault et al., 2020). Infection in wild hosts is typically asymptomatic, yet outbreaks in domestic pigs can cause nearly 100% mortality, leading to severe economic losses and making ASFV a major threat to global pig industries (Alonso et al., 2018; Galindo & Alonso, 2017; Gaudreault et al., 2020; Wang et al., 2021).

2.2.1.7. Rhabdoviridae

Rhabdoviruses are taxonomically complex, and their genomes typically have a negative-sense. Single-stranded RNA ranging from 10 to 16 kilobases encodes five core structural proteins (Dietzgen et al., 2017; Walker et al., 2015, 2018; Walker et al., 2022). Other classes have diverse accessory genes that contribute to their adaptability and host range. They have virions that are typically bullet-shaped or bacilliform and enveloped. These viruses infect a wide variety of hosts, including mammals, birds, reptiles, amphibians, fish, arthropods, and plants, with some species using arthropods as vectors for transmission to animals or plants (Dietzgen et al., 2017; Kuzmin et al., 2009; P. J. Walker et al., 2018). The most well-known member is Rabies lyssavirus, the principal cause of rabies in humans and animals, but the family also includes other important pathogens affecting livestock, fish, and crops (Shepherd et al., 2023; Walker et al., 2022; Walker et al., 2022). Recent advances in metagenomics and sequencing have uncovered many novel rhabdoviruses in both vertebrate and invertebrate hosts, especially a wide diversity of these Insect Specific Viruses (ISVs) in various insect hosts, such as mosquitoes, sand flies, leafhoppers, and bean bugs. Examples include newly discovered rhabdoviruses in mosquitoes from the Americas and Africa, as well as novel viruses in leafhoppers and bean bugs, many of which form distinct clades within the family and show no evidence of infecting vertebrates (Cholleti et al., 2018; da Silva Ferreira et al., 2020a; Guo et al., 2022; Jia et al., 2021; Kuzmin et al., 2009; Phumee et al.,

2021; Vasilakis & Tesh, 2015; Walker et al., 2015; Xiao et al., 2024). This expands our understanding of their biodiversity and evolutionary relationships.

2.2.1.8. Orthomyxoviridae

The Orthomyxoviridae family, best known for influenza viruses, also includes several tick-borne viruses, usually found in the Thogotovirus and Quaranjavirus genera. Key examples are Thogoto virus, Dhori virus, and Bourbon virus, all of which have been detected in ticks and, in some cases, are associated with human disease (Cler et al., 1983; Labuda & Nuttall, 2004; Maqbool et al., 2022; Savage et al., 2018). The lone star tick (*Amblyomma americanum*) transmits Bourbon virus and is shown to be associated with human illness in the United States (Savage et al., 2018). Recent metagenomic studies have found other orthomyxoviruses in ticks from various regions, including highly divergent Quaranjaviruses in *Rhipicephalus* ticks from Africa and novel orthomyxovirus-like sequences in ticks from China and Brazil, and this suggests a broader diversity and distribution than previously recognised (Blomström et al., 2019; Cholleti et al., 2016; Guo et al., 2022; Lin & Pascall, 2024; Zhang et al., 2023). These tick-borne orthomyxoviruses share structural and genetic features with other Orthomyxoviridae, such as segmented negative-sense RNA genomes, but are distinct from the classic influenza viruses (Cholleti et al., 2018; Cler et al., 1983). The zoonotic potential of some of these viruses is still being evaluated, but their presence in aggressive human-biting ticks highlights the importance of ongoing surveillance and research into their ecology and public health impact (Guo et al., 2022; Lin & Pascall, 2024; Zhang et al., 2023).

2.2.1.9. Mesoniviridae and Negeviruses

Mesoniviridae and Negeviruses are groups of insect-specific viruses (ISVs) that are primarily found in mosquitoes and other insects and are closely related to, but distinct from, classic arboviruses that infect both insects and vertebrates (Agboli et al., 2023; da Silva Ferreira et al.,

2020b; Silva et al., 2024; Supriyono et al., 2020; Vasilakis & Tesh, 2015). These viruses have a wide geographic distribution and have been detected in various mosquito species worldwide, including recent discoveries in Germany, Brazil, and Indonesia (Agboli et al., 2023; da Silva Ferreira et al., 2020b; Silva et al., 2024; Supriyono et al., 2020). Mesoniviridae, such as Yichang virus, and Negeviruses, like Daeseongdong and Dezidougou viruses, replicate only in insect cells and do not infect vertebrates, making them true ISVs (Agboli et al., 2023; Supriyono et al., 2020; Vasilakis & Tesh, 2015). Studies show that these ISVs can interact with medically important arboviruses within mosquito hosts, sometimes inhibiting the replication of arboviruses like Orthobunyavirus and Usutu virus, which may influence the mosquitoes' ability to transmit diseases (Agboli et al., 2023; Vasilakis & Tesh, 2015). High-throughput sequencing has revealed a remarkable diversity of these viruses in mosquito and midge populations, with many novel species still being discovered (da Silva Ferreira et al., 2020b; Silva et al., 2024). The presence of Mesoniviridae and Negeviruses in biting midges and male mosquitoes further highlights their broad host range among insects (da Silva Ferreira et al., 2020b; Silva et al., 2024). Their discovery has expanded understanding of viral evolution, vector competence, and the potential for using ISVs as biological control agents or vaccine platforms (Agboli et al., 2023; Vasilakis & Tesh, 2015b). Overall, Mesoniviridae and Negeviruses are important components of the insect virome, shaping the ecology of vector populations and possibly affecting the transmission dynamics of human and animal pathogens.

2.2.2 Insect-Specific Viruses

Viruses that can only infect insects and do not replicate in vertebrates are termed Insect-specific viruses (ISVs). These are diverse groups of viruses across different families. Recent advances in sequencing have led to the discovery of many new ISVs, especially in mosquitoes and other

dipterans, and they have been gaining attention for their roles in viral evolution, vector biology, and potential applications in controlling vector-borne diseases. ISVs have been found in many virus families, including Flaviviridae, Bunyaviridae, Mesoniviridae, Reoviridae, Rhabdoviridae, Togaviridae, and the newly recognised Negeviruses (Blitvich & Firth, 2015; Bolling et al., 2015; Calzolari et al., 2016; da Silva Ferreira et al., 2020b; Moonen et al., 2023; Vasilakis et al., 2013). Insect-specific flaviviruses (ISFs) are a well-studied group, with at least two major phylogenetic branches and a global distribution in mosquitoes, sandflies, and chironomids (Blitvich & Firth, 2015; Calzolari et al., 2016; Guzman et al., 2018). ISVs have also been detected in other insects such as sandflies, midges, and beetles, not just in mosquitoes. For example, novel ISVs such as Cicadella viridis iflavirus 1 (CvIfV1) and Cicadella viridis nido-like virus 1 (CvNiLV1) have been discovered in green leafhoppers, expanding the known host range beyond mosquitoes (Blitvich & Firth, 2015; da Silva Ferreira et al., 2020b; Käfer et al., 2019). High-throughput sequencing and metagenomics are key tools driving the discovery of new ISVs and mapping their evolutionary relationships (Agboli et al., 2019; Nouri et al., 2018).

2.2.2.1 Transmission and Host Restriction

ISVs are typically kept in insect populations through vertical (parent to offspring) and horizontal (between individuals) transmission, with some, like *Anopheles gambiae* densovirus (AgDNV), also capable of sexual transmission (Barik et al., 2016; Patterson et al., 2020; Peterson et al., 2024). Barriers at multiple stages of the viral life cycle, including entry, replication, and temperature sensitivity, restrict them from infecting vertebrates (Elrefaey et al., 2020; Halbach et al., 2017). Some ISVs can induce "superinfection exclusion," where infection with an ISV can block later infection by pathogenic arboviruses (Blitvich & Firth, 2015; Peterson et al., 2024).

2.2.2.2 Applications and Impact

ISVs can modulate the replication of pathogenic arboviruses, potentially reducing vector competence and disease transmission (Carvalho & Long, 2021; Halbach et al., 2017; Nouri et al., 2018). Some ISVs, such as the Nhumirim virus, have been shown to reduce West Nile virus transmission in co-infected mosquitoes (Nouri et al., 2018). Densoviruses are being explored as gene delivery tools for paratransgenesis in mosquitoes, offering new avenues for genetic control of vector populations (Agboli et al., 2019; Barik et al., 2016).

2.2.3. Global Burden and Historical Impact of Arboviruses

Arboviruses have had a profound and growing impact on global public health because they cause millions of infections annually, with significant morbidity, mortality, and economic costs. Their impact is felt especially in tropical and subtropical regions, and their burden is increasing due to urbanisation, globalisation, climate change, and the expansion of vector habitats (Beckham & Tyler, 2015a; Casals, 1972; Girard et al., 2020; Young, 2018).

2.2.3.1 Global Burden

Dengue, Zika, and Chikungunya are the most significant arboviruses globally, with dengue alone putting half the world's population at risk and causing 100–400 million cases and up to 20,000 deaths each year, with an economic burden ranging from $2.1 to $57.3 billion USD globally for dengue and yellow fever (Li et al., 2021; Paixão et al., 2018b; Thompson et al., 2020; Wallau et al., 2023). Seroprevalence studies show high rates of exposure; for example, pooled global seroprevalence is 38% for dengue, 25% for chikungunya, and 18% for Zika, with many infections being asymptomatic and underreported (Li et al., 2021; Nobre et al., 2025). Arboviruses have caused 29 outbreaks in 25 countries in Africa in the year 2023, resulting in nearly 20,000 confirmed cases and 820 deaths (Bangoura et al., 2025; Gedefie et al., 2025). Table 1 shows the burden of arboviruses in the regions where they were measured.

Table 1: A table showing the cases of some arboviruses and the notable regions where they were recorded.

Arbovirus	Annual Cases/Impact	Notable Regions	Citation
Dengue	100–400 million cases, 20,000 deaths	Global (tropics/subtropics)	(Li et al., 2021; Paixão et al., 2018b; Wallau et al., 2023)
Chikungunya	106,000 DALYs lost/year	Americas, Asia	(Li et al., 2021; Puntasecca et al., 2021)
Zika	44,000 DALYs lost/year, birth defects	Americas, Asia	(Li et al., 2021; Puntasecca et al., 2021)
Yellow Fever	Major outbreaks, high fatality	Africa, South America	(Bangoura et al., 2025; Gedefie et al., 2025)

2.2.3.2. Historical Impact and Trends

Arboviruses have caused unpredictable and sometimes explosive epidemics, such as the Zika outbreak in the Americas and repeated dengue surges in Asia and Latin America (Gould et al., 2017; Musso et al., 2018; Paixão et al., 2018a). Their spread is accelerated by global travel, urbanisation, and climate change, which expands the range of vectors like *Aedes aegypti* and *Aedes albopictus* (Mordecai et al., 2020; Robert et al., 2020; Wallau et al., 2023). Historically, research and surveillance have lagged, especially in Africa and parts of Asia, leading to underestimation of

the true burden and delayed outbreak response (Bangoura et al., 2025; Gedefie et al., 2025; Hungwe et al., 2024).

2.2.3.3. Challenges and Future Directions

Surveillance and reporting are often inadequate, with many cases going undetected due to asymptomatic infections and limited diagnostics (Gedefie et al., 2025; Li et al., 2021; Nobre et al., 2025). With few effective vaccines (only for yellow fever and dengue), and vector control being the main control strategy, though it is often insufficient, there's a need for innovative ways to manage and control arboviruses (Girard et al., 2020; Paixão et al., 2018a; Weaver et al., 2018). WHO and global partners are now prioritising improved surveillance, data sharing, and research to better predict, prevent, and control arboviral diseases (Bangoura et al., 2025; Thompson et al., 2020; Wallau et al., 2023).

2.2.4. Theories of Arboviral Spread and Emergence

The emergence and spread of arboviruses are shaped by a complex network of vector competence, host alternation, evolutionary constraints, and ecosystem dynamics. It is also known that arbovirus transmission occurs mainly through two interconnected cycles: the sylvatic (enzootic) and urban (epidemic) cycles, each with distinct ecological and evolutionary implications.

2.2.4.1. Vector Competence and Transmission Cycles

The term "vector competence" refers to the mosquito's (or other arthropod's) ability to get, support, and transmit an arbovirus. The vector's anatomical barriers (midgut, salivary glands) are usually overcome by viruses to establish infection, and this is influenced by the vector's genetics, microbiota, immune responses, and environmental factors like temperature and humidity. Transmission cycles include:

Horizontal transmission: The virus moves between vectors and vertebrate hosts during blood

feeding.

Vertical transmission: The virus passes from parent to offspring. **Venereal transmission:** The virus is transmitted between mating mosquitoes (sexual transmission).

Urbanisation, climate, and habitat changes can alter vector competence and the frequency of these cycles, increasing the risk of outbreaks (Agarwal et al., 2017; Agha & Tchouassi, 2022; Gómez et al., 2022; LaDeau et al., 2015; Visser et al., 2025).

2.2.4.2. Host Alternation and Evolutionary Constraints

For arboviruses to be successful, they need to replicate in both arthropod vectors and vertebrate hosts. This imposes evolutionary constraints that limit specialisation but promote genetic stability. This host alternation can slow adaptation but also enables cross-species transmission when ecological conditions change. For example, dengue and yellow fever viruses have adapted from sylvatic cycles involving non-human primates and forest mosquitoes to urban cycles involving humans and *Aedes aegypti* (Althouse et al., 2016; Weaver, 2005; Weaver & Vasilakis, 2009).

2.2.4.3. Biodiversity, Ecosystem Dynamics, and Transmission Networks

Biodiversity in tropical regions is usually high, and this provides reservoirs for sylvatic cycles, where arboviruses circulate among wild animals (often non-human primates) and forest-dwelling mosquitoes. Ecosystem changes such as deforestation, urban expansion, and climate change can disrupt these cycles, leading to spill over into human populations. The traditional view of discrete transmission cycles is evolving toward a network model, recognising the complexity and adaptability of arbovirus maintenance in nature (Diaz et al., 2013; Eastwood et al., 2020; Marklewitz & Junglen, 2019; Taylor-Robinson, 2024).

2.2.4.4. Sylvatic and Urban Cycles

1. **Sylvatic Cycle:** Arboviruses circulate between wild animals (e.g., non-human primates) and forest mosquitoes (e.g., *Haemagogus, Culex, Aedes* species). These cycles serve as reservoirs for viruses such as yellow fever, dengue, Zika, and chikungunya. Spillover occurs when humans enter forested areas or when bridge vectors feed on both humans and wildlife, leading to human infection (Althouse et al., 2016; Eastwood et al., 2020; Figueiredo, 2019; Li et al., 2022; Valentine et al., 2019; Weaver, 2005).
2. **Urban Cycle:** Once adapted, arboviruses can circulate between humans and urban-adapted mosquitoes (primarily *Aedes aegypti* and *Ae. albopictus*), leading to large-scale epidemics. Urbanisation, high human density, and suitable breeding sites facilitate these cycles. Some arboviruses can also spill back from urban to sylvatic cycles, complicating eradication efforts (Agha & Tchouassi, 2022; Althouse et al., 2016; Figueiredo, 2019; Weaver, 2005; Weaver & Vasilakis, 2009).

2.2.5 Disproportionate Burden of Arboviruses in Sub-Saharan Africa

In sub-Saharan Africa, the burden of disease caused by arboviruses is usually disproportionately high and often underrecognized, with significant impacts on public health, economies, and healthcare systems. This burden is typically driven by a combination of high transmission rates, limited surveillance, diagnostic challenges, and environmental changes that favour arboviral spread (Bangoura et al., 2024; Kayange et al., 2023; Mremi et al., 2021).

2.2.5.1 Key Findings on Burden and Prevalence

Systematic reviews show a huge body of serological work detailing exposure to multiple arboviruses in the region. For example, pooled IgG seroprevalence rates in sub-Saharan Africa are 23.7% for chikungunya, 22.7% for dengue, 22.6% for West Nile, 16.4% for yellow fever, 13.1%

for Zika, and 9.2% for Rift Valley fever (Bangoura et al., 2024). In Ethiopia, molecular prevalence in humans reached 38.4% (Gedefie et al., 2025). Again, this region is prone to frequent outbreaks, for example, in 2023 alone, 29 arboviral outbreaks were reported across 25 African countries, resulting in nearly 20,000 confirmed cases and 820 deaths (Bangoura et al., 2025). Notable outbreaks include tens of thousands of chikungunya cases in Sudan and significant Rift Valley fever activity (Ahmed et al., 2020). Documented affected populations are typically children and adults living in rural communities. The literature reveals that children are particularly affected, with pooled prevalence rates of 16% for dengue and 7% for chikungunya among paediatric patients, and this could be from little surveillance work done within that population (Kayange et al., 2023). Rural adults show high seropositivity, with 46.6% having antibodies to at least one arbovirus in a Kenyan study (Mease et al., 2011).

2.2.5.2 Drivers of Disproportionate Burden

In a region with limited resources and endemic with malaria, arboviral infections with acute febrile symptoms are often misdiagnosed as malaria due to overlapping symptoms and limited diagnostic capacity, leading to underestimation of the true burden (Ahmed et al., 2020; Bane et al., 2024). Also, many countries lack robust surveillance and laboratory infrastructure, making it difficult to detect and respond to outbreaks on time (Ahmed et al., 2020; Bangoura et al., 2024, 2025; Gedefie et al., 2025). Finally, climate change, urbanisation, and poverty increase vector habitats and human exposure, while health systems are still focused on malaria (Ahmed et al., 2020; Mordecai et al., 2020).

2.3. Culex Mosquitoes: Biology, Ecology, and Vectorial Capacity

Culex mosquitoes are important vectors throughout the world, with different species playing key roles in the transmission of arboviruses and filarial parasites, notably in sub-Saharan Africa (SSA).

The taxonomy of *Culex* is complex, with the *Cx. pipiens* complex, which includes *Cx. quinquefasciatus*, *Cx. pipiens pipiens*, and *Cx. pipiens molestus*, and other species such as *Cx. antennatus* and *Cx. poicilipes* being important in SSA (Aardema et al., 2020; Farajollahi et al., 2011; Haba & McBride, 2022; Nchoutpouen et al., 2019). These mosquitoes also have varied life cycles, behaviours, and habitat preferences, where they are often seen to have adapted to polluted urban environments, which is relevant for cities like Accra (Boerlijst et al., 2022; Krol et al., 2024a; Nchoutpouen et al., 2019). Their feeding preferences range from ornithophily (prefers birds) to mammals, making some species effective bridge vectors for zoonotic pathogens (Blom et al., 2024; González et al., 2020; Griep et al., 2024; Wehmeyer et al., 2024). Seasonal dynamics, longevity, and environmental factors such as temperature and urbanisation influence their population structure and vectorial capacity (Boerlijst et al., 2022; Liu et al., 2020; Moser et al., 2023; Padde et al., 2024). Vector competence is shaped by genetic, ecological, and microbiome factors, affecting the efficiency of pathogen transmission (Ferraguti et al., 2023; Madhav et al., 2024a; Muturi et al., 2016; Wang et al., 2025). Effective surveillance and control rely on ecological trapping methodologies, with trap type, site choice, temporal sampling, and ethical considerations being critical for robust vector monitoring (Buckner et al., 2025; Epstein et al., 2021; Nchoutpouen et al., 2019; Wilke et al., 2021).

2.3.1. Taxonomy and Key Species in SSA

Culex is a medically important group of mosquitoes and is diverse, with several species being vectors for diseases in SSA. Finding key species is crucial for effective surveillance and control of mosquito-borne diseases in the region.

2.3.1.1 Key Species in SSA

1. *Culex quinquefasciatus* is commonly called "Southern House Mosquito" and is an important vector for various diseases, including lymphatic filariasis and several arboviruses. This species thrives in diverse environments, including wastewater drains, ditches, and containers, making it a major public health concern (Gopalakrishnan & Veer, 2018). Furthermore, its adaptability and resistance to insecticides complicate control efforts. *Culex* is also known to transmit pathogens like lymphatic filariasis and viruses such as West Nile, Japanese encephalitis, and St. Louis encephalitis. Recently, *Culex* flavivirus, an insect-specific virus, has been associated with influencing the vector's efficiency in transmitting pathogenic viruses (Anakha et al., 2023; Bhattacharya et al., 2016; Gopalakrishnan & Veer, 2018; Kang et al., 2021).
2. The *Culex pipiens complex* is an umbrella term used to refer to mosquito species such as *Culex pipiens*, *Culex quinquefasciatus*, and their hybrids. These mosquitoes are significant vectors for various diseases such as West Nile virus and St. Louis encephalitis. As a complex, they show considerable genetic diversity and ecological adaptability, influencing their vector competence and distribution across different regions (Kang et al., 2021). Different forms of *Culex pipiens*, such as Cx. pipiens and *Cx. molestus*, display distinct ecological preferences, with *Cx. pipiens* found in rural areas and *Cx. molestus* in urban settings (Beji et al., 2017).
3. *Culex antennatus* and *Culex poicilipes* are two key mosquito species in sub-Saharan Africa. They are not just widespread, but they are also tough, and they thrive in different environments while playing a major role in spreading diseases. They are found across the tropics, from Mauritania and Sudan to Kenya and Egypt. They especially thrive around irrigated farmland, like rice fields, where water management and crop cycles can cause

their numbers to skyrocket (Chandler & Highton, 1975; Mint et al., 2017; Simsaa et al., 2021). *Culex antennatus* and *Cx. poicilipes* have been implicated as vectors of Rift Valley fever virus, with natural infections documented during outbreaks in Mauritania and proven vector competence in laboratory studies in Egypt (Mint et al., 2017; Turell et al., 2001). In Sudan, *Cx. antennatus* is among the most abundant indoor-resting *Culex* species, while both species are present in outdoor environments and show seasonal fluctuations in abundance (Simsaa et al., 2021). Their breeding is influenced by water depth, vegetation, and the stage of agricultural development, with *Cx. poicilipes* favouring reflooded fields and *Cx. antennatus* becoming more prevalent later in the crop cycle (Chandler & Highton, 1975). Although less studied than *Cx. pipiens* or *Cx. quinquefasciatus,* these species are of epidemiological significance due to their ability to spread arboviruses and their adaptability to changing environments (Chandler & Highton, 1975; Mint et al., 2017; Turell et al., 2001).

2.3.2. Larval Ecology and Urban Adaptation

Culex mosquitoes, including *Cx. quinquefasciatus* and *Cx. pipiens,* have been able to live in cities because their larvae can live in both nutrient-rich and low-quality water. In cities, their larvae are commonly found in a variety of stagnant water sources, including polluted drains, stormwater catch basins, and man-made containers close to human habitation (Chatterjee et al., 2024; Noori et al., 2015; Wang et al., 2021). Researchers have found that *Culex* larvae do best in water that has moderate levels of pollution and high levels of phosphate and nitrate. These chemicals speed up the growth of the larvae and make it more likely that they will survive (Noori et al., 2015). The cities and residential areas often provide a better larval habitat than green spaces, which means more eggs are laid (oviposition), leading to larval abundance, while adults may disperse to parks

and other green areas for survival (Krol et al., 2024). The presence and abundance of *Culex* larvae are influenced by abiotic factors such as water temperature, pH, rainfall, and seasonality, with higher temperatures and stable water bodies favouring larval proliferation (Bouaoud et al., 2024; Gardner et al., 2012; Oforka et al., 2024; Soh & Aik, 2021). Urban infrastructure, such as subterranean storms drain systems, can be important places for larvae to live, but how productive they may be depended on how much food and clean water is available (Wang et al., 2021). The microbial community in breeding waters, which includes bacteria like *Bacillus cereus*, can also attract eggs and help larvae grow, which makes *Culex* even more successful in urban areas (Chatterjee et al., 2024). This ecological flexibility allows *Culex* mosquitoes to exploit a wide range of urban water bodies, making them persistent and challenging vectors in city environments (Chatterjee et al., 2024; Krol et al., 2024; Noori et al., 2015).

2.3.3. Host Feeding Preferences

Culex mosquitoes have flexible and regionally variable host feeding preferences, which are shaped by both species' traits and local environmental factors. Some studies show that *Culex* species, such as *Cx. pipiens* and *Cx. quinquefasciatus* are often generalist feeders. Their actual host choices can shift significantly depending on geography, habitat, and host availability (Griep et al., 2024; Köchling et al., 2024; Wehmeyer et al., 2024). For example, in Europe, *Cx. pipiens* primarily feeds on birds, especially Passeriformes and Columbiformes, making them important vectors for bird-associated viruses like West Nile virus, but they may also feed on mammals, reptiles, and amphibians when these are abundant (Garcia-Rejon et al., 2010; Köchling et al., 2024; Rizzoli et al., 2015; Rodríguez-Valencia et al., 2025). The *Cx. pipiens* complex includes forms with different tendencies: some are more ornithophilic (bird-preferring), while others, such as the *Cx. molestus*

form, feed more readily on mammals, including humans, and hybrids can show indiscriminate feeding (Köchling et al., 2024; Wehmeyer et al., 2024b). Regional studies and meta-analyses reveal that even within a single species, feeding patterns can vary widely between populations, with local adaptation and host availability playing major roles (Garcia-Rejon et al., 2010; Griep et al., 2024b; Köchling et al., 2024). It has been observed that some *Culex* species shift from feeding on birds to mammals as the year progresses or as host populations fluctuate, and this is sometimes influenced by seasonal changes (Edman, 1974; Kent et al., 2009). This flexibility in host choice allows *Culex* mosquitoes to act as bridge vectors, transmitting pathogens from wildlife to humans and domestic animals, and underscores the importance of local surveillance to understand disease risk (Griep et al., 2024b; Köchling et al., 2024; Wehmeyer et al., 2024b).

2.3.4. Seasonal Dynamics and Longevity of Culex mosquitoes

Temperature and seasonality affect the development rates, survival, and reproductive traits of *Culex* species and other arthropods (Moser et al., 2023; Padde et al., 2024; Rucci et al., 2023; Wolkoff et al., 2023). Studies have shown that warmer temperatures improve development but may reduce adult longevity and reproductive fitness, and cooler temperatures increase the longevity of the adult population. In West Africa, where temperatures tend to be high, this reveals an implication for vectorial capacity, potentially with an uptick in infectious cases (Moser et al., 2023; Padde et al., 2024; Wolkoff et al., 2023). Light pollution and urbanisation can disrupt seasonal diapause and biting seasons (Andreadis et al., 2014; Epstein et al., 2021; Wolkoff et al., 2023).

2.3.5. Vector Competence

The ability of a mosquito to acquire, maintain, and transmit a pathogen is referred to as vector competence, and a complex interplay of intrinsic and extrinsic factors shapes this ability (Lewis et

al., 2023). Some intrinsic factors that shape this ability include the mosquito's genetic background, which determines susceptibility to infection, the effectiveness of immune responses, and physical and cellular barriers within the mosquito, such as the midgut and salivary glands, that viruses must overcome to be transmitted (Beerntsen et al., 2000; Hardy et al., 1983; Lewis et al., 2023). There are four of these barriers: midgut infection (overcoming the midgut infection barrier, MIB), midgut escape into the hemocoel (midgut escape barrier, MEB), salivary gland infection and replication (salivary gland infection barrier, SIB), and virion release into saliva (salivary gland escape barrier, SEB) (Carpenter & Clem, 2023; Stauft et al., 2022). The mosquito's microbiota, which is made up of symbiotic bacteria and insect-specific viruses, can either enhance or inhibit pathogen replication and transmission (Chen et al., 2023; de Faria et al., 2024; Lewis et al., 2023). The microbiome is largely made up of Proteobacteria and Wolbachia. Different strains have different effects on different species of mosquitoes. A study found that natural high-density infection in Ben9 *Cx. quinquefasciatus* results in a 52% drop in WNV body infection, while artificial wAlbB in *Cx. tarsalis* resulted in a 25% rise in WNV infection rate (Dodson et al., 2014; Micieli & Glaser, 2014). Environmental factors such as temperature, mosquito age, and the viral dose in the blood meal also play significant roles. For example, at higher temperatures, viral replication is accelerated, and the incubation period is shortened. Older mosquitoes may be more susceptible depending on species and environmental context (Richards et al., 2009, 2010; Vogels et al., 2017). Additionally, differences between mosquito populations or colonies, even within the same species, can lead to substantial variation in vector competence due to local adaptation and genetic diversity (Richards et al., 2009, 2010; Vogels et al., 2017). Insecticide resistance may alter mosquito immunity and microbiota, potentially increasing susceptibility to pathogens, though evidence is mixed and context-dependent (Suh et al., 2023). Ultimately, the efficiency of transmission is

determined by the interaction of these factors, which together influence whether a mosquito can successfully transmit a pathogen to a new host (Beerntsen et al., 2000; Hardy et al., 1983; Kain et al., 2022; Lewis et al., 2023; Vogels et al., 2017).

2.3.6. Trap Types and Site Selection

CDC light traps, BG-Sentinel, gravid traps, and resting boxes are commonly used for *Culex* surveillance, each with varying effectiveness depending on species and habitat (Buckner et al., 2025; Nchoutpouen et al., 2019; Wilke et al., 2021). Site selection strategies must consider urban versus rural settings, habitat types, and microclimatic conditions to improve trap yields (Krol et al., 2024; Nchoutpouen et al., 2019; Wilke et al., 2021).

2.4. Arboviral Surveillance

Monitoring and controlling mosquito-borne diseases relies heavily on surveillance of arboviruses transmitted by *Culex* and *Aedes* mosquitoes. Although both genera are important vectors, their surveillance systems differ in several important aspects, including target viruses, sampling strategies, diagnostic approaches, and data integration. Dengue, Zika, and chikungunya are among the viruses checked for in *Aedes* mosquitoes, which employs intensive, site-specific monitoring and rapid molecular diagnostics to detect outbreaks early (Reis et al., 2019; Lühken et al., 2023; de Melo et al., 2024; Seang-arwut et al., 2023; Wilke et al., 2019). In contrast, *Culex* surveillance is typically broader in geographic scope, targeting rural and peri-urban areas and focusing on viruses like West Nile, Japanese encephalitis, and Usutu, with an emphasis on long-term ecological monitoring and integration with animal and avian surveillance (Calzolari et al., 2022, 2024; Fang, Li, et al., 2021; Holicki et al., 2020; Rothman et al., 2021). There is also a difference in data structures and reporting systems, with *Aedes* surveillance characterised by real-time outbreak-driven data flows, while *Culex* systems may rely on seasonal or sentinel-based reporting (Calzolari

et al., 2022, 2024; Wipf et al., 2019). Recent advances, such as automated mosquito identification and metagenomic sequencing, are being applied to both systems, but with varying degrees of adoption and focus (González-Pérez et al., 2024; Motta et al., 2019, 2020; Shi et al., 2019). Despite some overlap, these differences reflect the unique epidemiology, ecology, and public health impact of the arboviruses each genus transmits. Understanding these distinctions is critical for improving surveillance and response strategies (Epelboin et al., 2017; Fang, Li, et al., 2021; Fang, Zhang, et al., 2021; Izquierdo-Suzán et al., 2024; Lühken et al., 2023).

2.4.1. Integrated Surveillance

Integrated surveillance is the strategic blend of multiple monitoring streams, such as human, animal, environmental, laboratory and socio-behavioural, into an interoperable system that detects, analyses and triggers responses to health threats faster and more efficiently than siloed, disease-specific ("vertical") approaches (Fall et al., 2019). These systems are needed for early detection and control of zoonotic diseases, such as Rift Valley Fever Virus (RVFV) and Japanese Encephalitis Virus (JEV). In sub-Saharan Africa (SSA), human syndromic surveillance and animal surveillance have been challenging, making integration and adaptation of these systems critical.

2.4.2 Human Syndromic Surveillance: Limitations in SSA

Resource constraints are still a significant roadblock in SSA with traditional surveillance relying on laboratory confirmation, and this makes it impractical in SSA due to limited resources, infrastructure, and trained personnel. Syndromic surveillance uses clinical symptoms rather than lab results, and this offers a cost-effective and timely alternative but may lack specificity and comprehensive coverage (Mwanja et al., 2024a). Some studies show that less than 70% of weekly reporting in multiple SSA Integrated Disease Surveillance Report reviews results in

missed temporal clusters of febrile/neurological syndromes. Again, reports reach the national level with a median of 14-26 days' delay, leading to outbreak signals being diluted and lagging behind vector control. There is also an overlap with malaria, dengue, bacterial meningitis and arboviral neuroinvasive disease, which sometimes causes a high false-positive rate and resource fatigue, and less than 40% of district labs can test arboviruses, creating a weak feedback loop (Chretien et al., 2008; Mremi et al., 2021; Mwanja et al., 2024; Scales, 2020; Wolfe et al., 2021).

2.4.3. Animal surveillance includes sentinel animals, wildlife, and livestock monitoring.

Various studies placed different sentinel animals (healthy animals placed in populations to detect infection), like chickens, pigs, and small ruminant herds, for various viruses. The frequency was 6–12 birds/flock, bled biweekly from June to October; 5–20 piglets per site, and bled every 2 weeks, and 10–20 sheep/goats per site; IgM ELISA monthly from August to November. The results revealed that they were all able to detect infection within 2–4 weeks pre-human case window (Amdouni et al., 2020; Barry et al., 2022; Borah et al., 2012; Chevalier et al., 2007; FAO, 2003; Langevin et al., 2001; Mhamadi et al., 2023; Tamba et al., 2024).

2.5. Entomological (vector) surveillance

Entomological (vector) surveillance is a cornerstone of public health efforts to control mosquito-borne diseases like malaria and arboviruses. In sub-Saharan Africa (SSA) and Ghana, effective vector monitoring is critical but faces significant challenges, especially as disease priorities shift and resources are still limited (Amoa-Bosompem et al., 2020; Kouassi et al., 2023).

2.5.1 Importance of Entomological Monitoring

Early Warning & Control: Monitoring vector species, abundance, and infection rates enables timely interventions and guides vector control strategies, such as targeted insecticide use and

distribution of bed nets (Afrane et al., 2024; Amoa-Bosompem et al., 2020; Coleman et al., 2023; Kouassi et al., 2023; Kudom, 2020).

Species Identification: Accurate identification, including cryptic and invasive species (e.g., *Anopheles stephensi*), is essential for understanding transmission dynamics and emerging risks (Afrane et al., 2024; Amoa-Bosompem et al., 2020; Ismail et al., 2024).

2.5.2 Traditional Methods

Species ID & Abundance: Morphological and molecular techniques are used to identify mosquito species and estimate their population densities (Afrane et al., 2024; Amoa-Bosompem et al., 2020; Coleman et al., 2023; Mwalimu et al., 2024).

Infection Rates: PCR and virus isolation are standard for detecting pathogens in vectors, providing direct evidence of transmission risk (Amoa-Bosompem et al., 2020; Mojica et al., 2025).

Larval Indices: House, container, and Breteau indices help estimate breeding site abundance and outbreak potential (Kudom, 2020; Ouattara et al., 2019).

2.6. Xenodiagnostics: Concept and Limitations

Xenodiagnostics involves using live vectors to detect pathogens in hosts. Historically, it has been used for diseases like Chagas, but it is rarely applied in mosquito surveillance due to ethical and practical concerns (Cameron & Ramesh, 2021; Marques et al., 2014; Pryce et al., 2021; Pryce & Reimer, 2021).

Ethical issues (exposing vectors/hosts), logistical complexity, and limited scalability make it impractical for routine surveillance in SSA.

2.6.1 Challenges in SSA/Ghana

Resource Constraints: Limited funding, infrastructure, and skilled personnel hinder comprehensive surveillance (Agboli et al., 2022; Amoa-Bosompem et al., 2020; Buchwald et al., 2020; Fournet et al., 2018).

Diagnostic Gaps: Weak laboratory capacity restricts detection of arboviruses and exact vector identification (Agboli et al., 2022; Amoa-Bosompem et al., 2020).

Fragmented Data Systems: Lack of integrated, harmonised data impedes timely response and coordinated action (Agboli et al., 2022; Fournet et al., 2018).

Disease Prioritisation: Malaria receives most resources, often at the expense of arbovirus surveillance, despite rising arboviral threats (Agboli et al., 2022; Buchwald et al., 2020; Mordecai et al., 2020).

2.7. The Ghanaian Context: Arboviruses, Culex, and Surveillance Gaps

Ghana has a diverse ecosystem, comprising coastal, forest, and savannah, and this creates varied risks for arboviral transmission, with urban centres like Accra and rural northern areas such as Navrongo facing different vulnerabilities. The surveillance of arboviruses and their vectors, especially *Culex* mosquitoes, is crucial but remains limited in scope and effectiveness.

2.7.1. Ecological Diversity and Arboviral Vulnerability

Ghana's diverse ecological zones support different mosquito populations, leading to risks in arbovirus transmission. Urban and coastal areas (e.g., Accra) differ from savannah regions (e.g., Navrongo) in vector species composition and abundance, influencing local vulnerability to outbreaks.

2.7.2. Evidence of Arbovirus Circulation

2.7.2.1. Seroprevalence

Over the years, arboviral infections in Ghana have primarily been identified through serological assessments that detect IgM and IgG antibodies in patient samples. These methods, while useful for confirming prior exposure, fall short of confirming active infections. Many seroprevalence surveys have shown that the yellow fever virus (YFV) and the dengue virus (DENV) are circulating within the country (Agboli et al., 2022). However, for viruses like West Nile (WNV), chikungunya (CHIKV), and Rift Valley fever (RVFV), evidence is either anecdotal or absent, and no large-scale seroprevalence studies have confirmed their presence in the Ghanaian population.

2.7.2.2. Outbreaks

Yellow fever has been the most documented arbovirus in Ghana's public health history, with several outbreaks reported over the decades. Yet, major epidemics have not been recorded in recent years. Despite the ecological suitability and presence of competent mosquito vectors, dengue fever had remained officially unconfirmed in Ghana until recently. In an update, the Ghana Health Service issued a health alert in 2024 that confirmed laboratory-verified cases of dengue fever for the first time (HEALTH ALERT ON DENGUE FEVER—Ministry of Health, 2024). Conversely, there is currently no evidence of Japanese encephalitis virus (JEV) circulation in Ghana (WHO, 2025).

2.7.2.3. Virus Detection

Despite Ghana's favourable ecological conditions for mosquito-borne viruses, it has proven difficult to isolate or find these viruses directly from mosquito vectors. According to Amoa-Bosompem et al. (2020), a comprehensive study that involved trapping mosquitoes across several ecological zones and screening them for known arboviruses did not detect any health-threatening

arboviruses in the mosquito samples tested (Amoa-Bosompem et al., 2020). This finding reflects a broader trend: while serology has consistently hinted at human exposure to arboviruses, actual virus detection and molecular characterisation from either vectors or hosts remain limited in Ghana. This disconnect underscores a critical gap in the country's arboviral surveillance system. The current reliance on serological evidence without parallel entomological or virological confirmation poses a risk of underestimating the true burden and transmission dynamics of arboviruses. Strengthening diagnostics, particularly molecular tools, and vector screening protocols will be key to building a more responsive and proactive public health system capable of offering early warning for future arboviral outbreaks.

2.7.3. *Culex* Species Distribution and Abundance

Culex mosquitoes are widespread in Ghana, but detailed mapping of their distribution and abundance is lacking. Studies emphasise the complexity of the *Culex* virome and the need for enhanced surveillance (Ha et al., 2021; Madhav et al., 2024b).

2.8. Traditional and Modern Methods for Viral Detection in Mosquitoes

Methods for detecting viruses in mosquitoes have evolved from traditional cell culture and immunoassays to advanced molecular and metagenomic techniques in arbovirus surveillance and vector control. The gold standard for virus isolation has historically been cell culture owing to its specificity, but it is limited by time, cost, and biosafety (Huang et al., 2022; Rosen & Gubler, 1974; Tesh, 1979). ELISAs and IFAs are rapid and scalable alternatives but can suffer from cross-reactivity and limited sensitivity (Burkot et al., 1984; O'Brien et al., 2015; Sánchez-Purrà et al., 2017). By providing high sensitivity and specificity for known viruses, targeted molecular assays, such as RT-PCR and qRT-PCR, have revolutionised surveillance. Despite this, these methods are limited by the inability to detect novel or unexpected pathogens and by the bias of primers

(Avramov et al., 2024; Faye et al., 2013; Hadfield et al., 2001; Koliopoulos et al., 2021; Peper et al., 2024; Tang et al., 2020b; Wasieloski et al., 1994). Serotyping and genotyping through sequencing of amplicons further enhance the resolution of these assays (Balingit et al., 2020; Faye et al., 2013).

Metagenomics using next-generation sequencing (NGS) approaches enables virome profiling, discovery of novel viruses, and an understanding of virus-mosquito-microbiome interactions (Koh & Saleh, 2024; Vieira et al., 2024; Williams et al., 2022). High-throughput NGS platforms (e.g., Illumina, Oxford Nanopore) and advanced bioinformatics pipelines enable rapid, cost-effective, and field-deployable surveillance, especially when combined with multiplex PCR, microfluidics, and artificial intelligence (Koh & Saleh, 2024; Vieira et al., 2024; Williams et al., 2022).

2.9. The Value of Virome Data

Virome data can offer benefits beyond simply discovering pathogens in mosquitoes. By analysing the full spectrum of viruses, including insect-specific viruses (ISVs), researchers might gain insights into mosquito biology, ecology, and public health, supporting both vector control and broader "One Health" initiatives. It is known that ISVs may have an influence on mosquito immune responses. This may have an impact on their ability to transmit human pathogens, offering clues for vector competence and potential biocontrol strategies, and this dynamic can be revealed through virome data analysis (Wu et al., 2023). Again, it is noteworthy that the virome composition may vary by mosquito species, location, and season. For example, studies show that viral diversity and community structure are highly dependent on mosquito species and habitat, with certain viruses specific to environments (e.g., urban vs. forested areas). This helps track how ecological factors shape virome diversity and potential disease risk (Wu et al., 2023). Also, ISVs identified in virome studies may serve as natural biocontrol agents, reducing the prevalence or transmission

of harmful arboviruses (Wu et al., 2023). Comprehensive virome data helps clarify how the presence of ISVs or other viruses may enhance or suppress a mosquito's ability to transmit pathogens, informing risk assessments and intervention strategies (Wu et al., 2023). Virome sequencing builds robust reference databases, improving the accuracy and speed of future diagnostics and surveillance efforts (Kwok et al., 2020; Schuele et al., 2020; Wu et al., 2023). Virome data support integrated surveillance at the human-animal-environment interface, enabling early detection of emerging threats and informing public health responses (Kwok et al., 2020; Matos et al., 2025; Schuele et al., 2020; Tiu et al., 2022; J. R. Walker et al., 2025; Q. Wu et al., 2023).

CHAPTER THREE

3.0. METHODS

3.1. Study Design

This was a cross-sectional study that incorporated retrospective analysis of previously collected adult *Culex* mosquitoes from Teshie Police Barracks, Burma Camp, 37 Military Barracks—Accra (collected from June 2022 to August 2022), and Bonia, Korania, Navrongo Health and Medical Research Centre (NHRC), Chiana, and Nogsenia—Navrongo (collected from January 2020 to December 2020). It also included a prospective analysis of *Culex* species collected from the University of Ghana campus using larval collections and Biogents BG-Pro CDC light traps, and Cantonments catch using BG-Pro CDC light traps. These were collected within the period of August 2024 to November 2024.

3.2. Ethical and Scientific Approval

Scientific approval was sought from the Scientific and Technical Committee of the Noguchi Memorial Institute for Medical Research, with an STC number of STC Paper 2(2) 25-26.

3.3. Sample Collection

Biogents BG-Pro CDC light traps were set up at 4:30 PM and retrieved at 8:00 AM the next morning. Larvae were collected with ladles and dippers from gutters and abandoned car tyres and transported to the Noguchi Memorial Institute for Medical Research to mature into adults.

3.4. Sample Processing

3.4.1. *Culex* Identification and Speciation

Prospective adult mosquitoes recovered from the Biogents BG-Pro CDC light traps and adults from mature larvae, as well as mosquito pools from retrospective collections from Teshie Police

Barracks, Burma Camp, and 37 Military Barracks, were identified and speciated using morphological keys available at the Walter Reed Biosystematics Unit (https://wrbu.si.edu).

3.4.2. Total Nucleic Acid Extraction

After speciating the mosquitoes according to species and collection sites, they were pooled 1 to 20 mosquitoes and transferred to 1.5 mL Eppendorf tubes. Each pool was placed in a 1.5 mL tube, and 560 µL of prepared Buffer AVL (to lyse the cells and expose the virus) was pipetted into it and crushed using sterilised plastic pestles. For the extraction, the QIAamp Viral RNA extraction kit (Qiagen) was used. After crushing the mosquitoes, they were incubated at room temperature (15–25°C) for 10 minutes. Afterwards, the tubes were briefly centrifuged to remove drops from the inside of the lid. A volume of 560 µL of cold ethanol (96–100%) was added to the sample to precipitate the nucleic acid. The mixture was then vortexed for 15 seconds to ensure a homogenous mixture and then briefly centrifuged to remove drops from inside the lid. Carefully, 600 µL of the previously prepared solution was transferred to the QIAamp Mini spin column (in a 2 mL collection tube) without wetting the rim. The sample was then centrifuged at 8000 rpm for 1 minute. The QIAamp Mini column was then placed into a clean 2 mL collection tube, and the filtrate was discarded. The QIAamp Mini column was carefully opened, and the remaining solution was transferred (in a 2 mL collection tube) without wetting the rim again.

The QIAamp Mini column was carefully opened, and 500 µL of Buffer AW1 was added to denature the proteins present in the pools. Then, the cap was closed and centrifuged at 8000 rpm for 1 minute. After that, the QIAamp Mini column was placed in a clean 2 mL collection tube, and the tube containing the filtrate was discarded. Next, the QIAamp Mini column was carefully opened, 500 µL of Buffer AW2 was added to remove residual salts, and it was centrifuged at full speed (14,000 rpm) for 3 minutes. Following that, the QIAamp Mini column was placed in a clean

1.5 mL microcentrifuge tube, and the old collection tube containing the filtrate was discarded. Subsequently, the QIAamp Mini column was carefully opened, and 80 µL of Buffer AVE was added to solubilise the extracted nucleic material into solution for downstream analysis. Then, the cap was closed and incubated at room temperature for 1 minute. Afterwards, it was centrifuged at 8000 rpm for 1 minute to obtain the extracted nucleic acid. The extracts were then stored at -80°C, pending further analysis.

3.4.3. Molecular detection of Mosquito-borne pathogens

3.4.3.1. Detection of Pan-flavivirus

The Pan-flavivirus was detected by polymerase chain reaction (PCR) using the primers (VD8 – 5' – GGG TCT CCT CTA ACC CTC AG – 3' and EMF1 – 5' – TGG ATG ACS ACK GAR GAY ATG) targeting the NS5 gene, using the protocols designed by Vazquez and Daidoji with modifications (Daidoji et al., 2021; Vázquez et al., 2012). The PCR was conducted using the thermocycler (Eppendorf *Mastercycler X50s*), 2% gel prepared in Tris-Acetate EDTA (TAE) buffer, and Qiagen One-Step RT-PCR kit (Qiagen, Hilden, Germany). The PCR components were placed on a cold rack before the addition of the RNA extracts and reagents. The master mix (12.5 µL) was pipetted into the wells of the 0.2 mL semi-skirted 96-well PCR plate. The plate was set up with a positive control template using available extracted RNA from Dengue virus and a non-template control using nuclease-free water. To avoid cross-contamination, the master mix was prepared in a designated PCR workstation for master mix preparation only. Table 2 shows the master mix for the reaction, and the thermocycler conditions set are as found in Table 3.

Table 2: Master mix components for PCR Pan-flavivirus RNA template

Reagents	Volume/tube (µL)
Nuclease-free water	5
5x Reaction Buffer	5
Q solution	5
dNTP mix	1
EMF1	1.5
VD8	1.5
Enzyme Mix	1
Template RNA	5
Total Reaction Volume	25

Table 3: PCR reaction steps and cycling conditions.

	Temp/Time	Number of Cycles
Stage 1: Reverse Transcriptase	50°C / 30 mins	1
Stage 2: Hot start	95°C / 15 mins	1
Stage 3: Denaturation	95°C / 30 sec	45
Annealing and extension	52°C / 30 sec	
	72°C / 30 sec	
Final Step	30°C / 30 sec	1

3.4.3.1.2. Detection of Alphavirus

The Alphavirus was detected by a nested PCR using the primers targeting the RNA-dependent RNA polymerase gene. The PCR was conducted using the thermocycler (Eppendorf *Mastercycler X50s*), 2% gel prepared in Tris-Acetate EDTA (TAE) buffer, Qiagen One-Step RT-PCR kit for the step one (1) reaction with the primers (ALPHA 500 F-TTT AAG TTT GGT GCG ATG ATG AAG TC & ALPHA 500 R-GCA TCT ATG ATA TTG ACT TCC ATG TT) and Taq reaction buffer, Taq polymerase, dNTPs, nuclease-free water and primers for step two (2) (Biolab 10x Standard Taq reaction buffer) using the primers (ALPHA 500 F-TTT AAG TTT GGT GCG ATG ATG AAG TC, ALPHA 100 F-CTA TGA TAT TGA CTT CCA TGT TCA TCC & ALPHA 100 R-CTA TGA TAT TGA CTT CCA TGT TCA GCCA). The PCR components were placed on a cool rack before the addition of the RNA extracts and reagents. The master mix (I2.5 µL) was pipetted into the wells of the 0.2mL semi-skirted 96-well PCR plate. The plate was set up with a positive control template and a non-template control. To avoid cross-contamination, the master mix was prepared in a designated hood for master mix preparation only. Tables 4 and 5 show the master mix for the reaction at steps 1 and 2, respectively, and the thermocycler conditions set are as found in Table 4.

Table 4: Master mix components for Step 1 RT-PCR kit Alphavirus RNA template

Reagents	Volume/tube (µL)
Nuclease-free water	5
5x Qiagen One-Step Buffer	2.5
dNTP mix	0.5
Alpha 500F	0.75
Alpha 500R	0.75
Enzyme Mix	0.5

Template RNA	2.5
Total Reaction Volume	12.5

Table 5: Master mix components for Step 2 using Biolab 10x Standard Taq Reaction Buffer

Reagents	Volume/tube (µL)
Nuclease-free water	15.875
Standard Taq reaction Buffer	2.5
dNTP mix	0.5
Alpha 500F	0.5
Alpha 100F	0.25
Alpha 100R	0.25
Taq Polymerase	0.125
Template RNA	5
Total Reaction Volume	25

Table 6: PCR reaction steps and cycling conditions for Step 1.

	Temp/Time	Number of Cycles
Stage 1: Reverse Transcriptase	45°C / 15 mins	1
Stage 2: Hot start	95°C / 3 mins	1
Stage 3: Denaturation	95°C / 20 sec	10
Annealing and extension I	65°C / 30 sec (decrease by 1°C after each step)	
	72°C / 20 sec	

Stage 4: Denaturation	95°C / 20 sec	30
Annealing and extension II	55°C / 20 sec	
	72°C / 20 sec	

Table 7: PCR reaction steps and cycling conditions for Step 1.

	Temp/Time	Number of Cycles
Stage 1: Hot start	95°C / 2 mins	1
Stage 2: Denaturation	95°C / 20 sec	40
Annealing and extension I	58°C / 20 sec	
	72°C / 20 sec	

3.5. Library Preparation, Sequencing and Bioinformatics Analysis

Twenty (20) pools of the *Culex* extract were selected at random, including 12 from Accra and 8 from Navrongo. Qubit was conducted on the selected pools to determine the nucleic acid concentration (quantification). To obtain complementary DNA (cDNA), the RNA was retrotranscribed with SuperScript IV reverse transcriptase using random hexamers (Invitrogen, Vilnius, Lithuania). Random amplification of cDNAs were performed using the multiple displacement amplification (MDA) protocol with phi29 polymerase and random hexamers (Nelson, 2014). Libraries were sequenced at a depth of 60 to 80 million reads on an Illumina NextSeq2000 platform in a 150-base pair (bp) single-read format.

Raw paired-end Illumina reads (150 bp) from *Culex* mosquito samples were subjected to quality control using FastQC (Andrews, 2010) to assess base quality, GC content, adapter contamination, and sequence duplication. Adapters and low-quality bases were trimmed using fastp v0.23.2 (Chen, 2025). Bases with a Phred score <20 were trimmed and reads shorter than 50 bp after trimming

were discarded. High-quality trimmed reads in FASTQ format. To enrich for viral sequences, host-derived reads (*Culex quinquefasciatus*) were removed by alignment to the reference genome (Assembly: GCF_015732765.1) using Bowtie2 v2.5.1 (Langmead & Salzberg, 2012). Reads were aligned, and non-host reads were extracted. Host-depleted reads were taxonomically classified using Kraken2 v2.1.2 (Wood et al., 2019) with a custom viral database 0.5 for minimum classification confidence. Reads classified as viral or unclassified were assembled *de novo* using SPAdes v3.15.5 (Bankevich et al., 2012). Consensus sequences were generated from viral contigs (>1,500 bp) and used for downstream analysis. Consensus viral sequences were annotated by homology search against the NCBI nt database using BLASTn v2.13.0+ (Camacho & Madden, 2025). The significance threshold was set to E-value < 1×10^{-10}. DNA sequences were aligned using the Clustal Omega algorithm implemented in the msa package in R (R Core Team, 2025). The multiple sequence alignment was converted to a phyDat object, and a maximum likelihood (ML) phylogenetic tree was inferred using the JC69 nucleotide substitution model with the phangorn package (Schliep, 2011). Bootstrap analysis was performed with 1,000 replicates to assess branch support. The resulting tree was exported in Newick (.nwk) format. The tree was subsequently visualised and annotated in FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/), where bootstrap support values were displayed at corresponding nodes

CHAPTER FOUR

4.0. RESULTS

4.1 Culex Distribution

A total of 2,886 *Culex* mosquitoes were collected and identified across various sites, including those captured as adults from mature larvae. These were organised into 217 pools, each comprising between one and twenty mosquitoes, depending on the species and collection site.

Of these pools, 37 originated from Navrongo and 180 from urban Accra. Among the Accra samples, 39 pools were prospective collections (from the University of Ghana and Cantonments), while 178 were archived samples (from Navrongo, Teshie Police Barracks, Burma Camp, and 37 Military Barracks).

The most abundant species were *Culex pipiens* and *Culex quinquefasciatus*, together accounting for 1,758 mosquitoes (60.3% of the total catch). In Navrongo, only *Culex quinquefasciatus* was recorded, with a total of 382 mosquitoes. Because the Walter Reed Biological Keys could not reliably distinguish *Culex pipiens* from *Culex quinquefasciatus*, these species were grouped for analysis. Thus, species distribution profiles were calculated separately for Accra (prospective and archived collections).

In Accra, *Culex pipiens* and *Culex quinquefasciatus* comprised 1,376 mosquitoes (55%) of the total. A considerable proportion (632 mosquitoes, 25.2%) was classified as unidentified *Culex* due to damage to key morphological features. Other notable species included:

- *Culex antennatus* – 228 (9.1%)
- *Culex pruina* – 154 (6.1%)
- *Culex neavei* – 71 (2.8%)
- *Culex tritaeniorhynchus* – 31 (1.2%)

Rarely collected species included *Culex zombaensis* – 10 (0.4%) and *Culex univittatus/perexiguus* – 1 (0.04%). Figure 1 shows the distribution of the mosquito species collected.

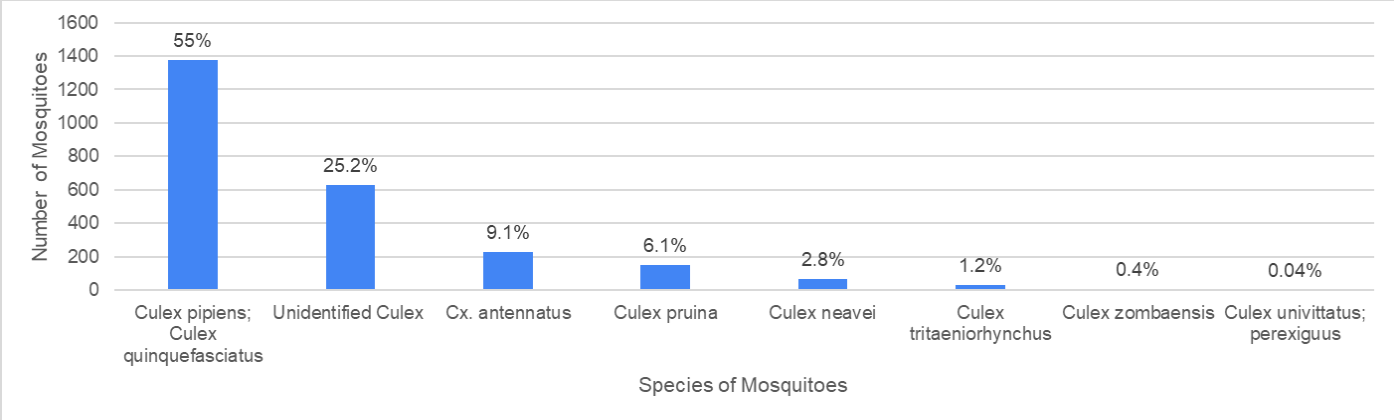

Figure 1: A histogram of the various Culex species identified from Accra.

4.2. Blood Feeding Distribution

Figure 2 shows the blood-feeding distribution of the *Culex* collected from all sites, including Navrongo. The majority of the *Culex* collected were non-blood-fed (73%), while the blood-fed mosquitoes accounted for 27% of the total mosquitoes.

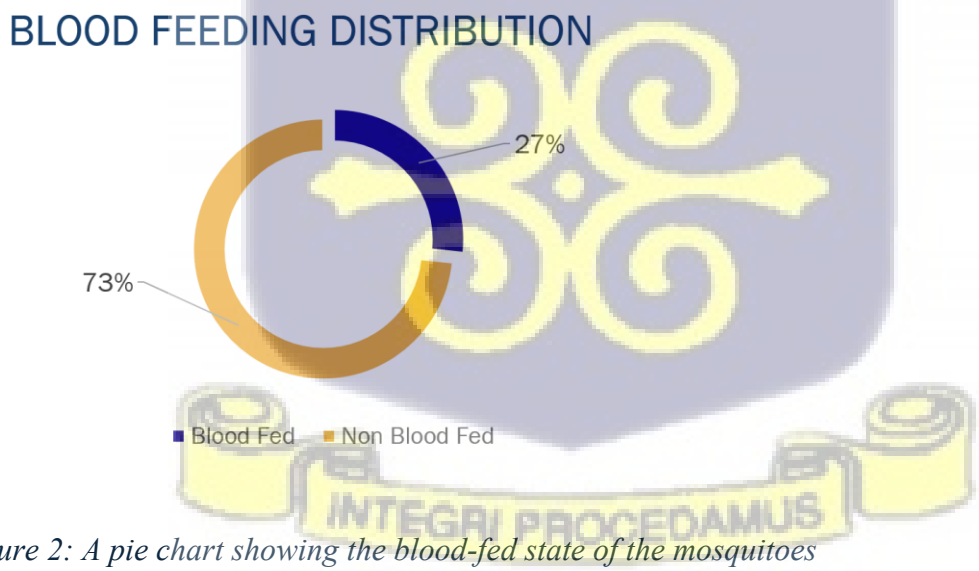

Figure 2: A pie chart showing the blood-fed state of the mosquitoes

4.3. A gel image Pan-flavivirus PCR

All 217 pools subjected to conventional PCR of Pan-flavivirus showed with this PCR gel electrophoresis image, as there was no amplification in all the wells, except the positive control. Indicating that the samples had no flavivirus. As shown in Figure 3, the well labelled M is the ladder, P is the positive control at 610 bp, and N is the negative control. Wells 1 – 22 are samples.

Figure 3: A gel image of the samples after the Flavivirus PCR was run

4.4. Gel image of Pan-alphavirus PCR

All 217 pools subjected to conventional PCR for Pan-alphavirus showed no amplification in any of the wells, except for the positive control. This indicates that the samples did not contain alphavirus. As seen in Figure 4, the well labelled M is the ladder, P is the positive control at 210 bp, and N is the negative control. Wells 1 – 24 are samples

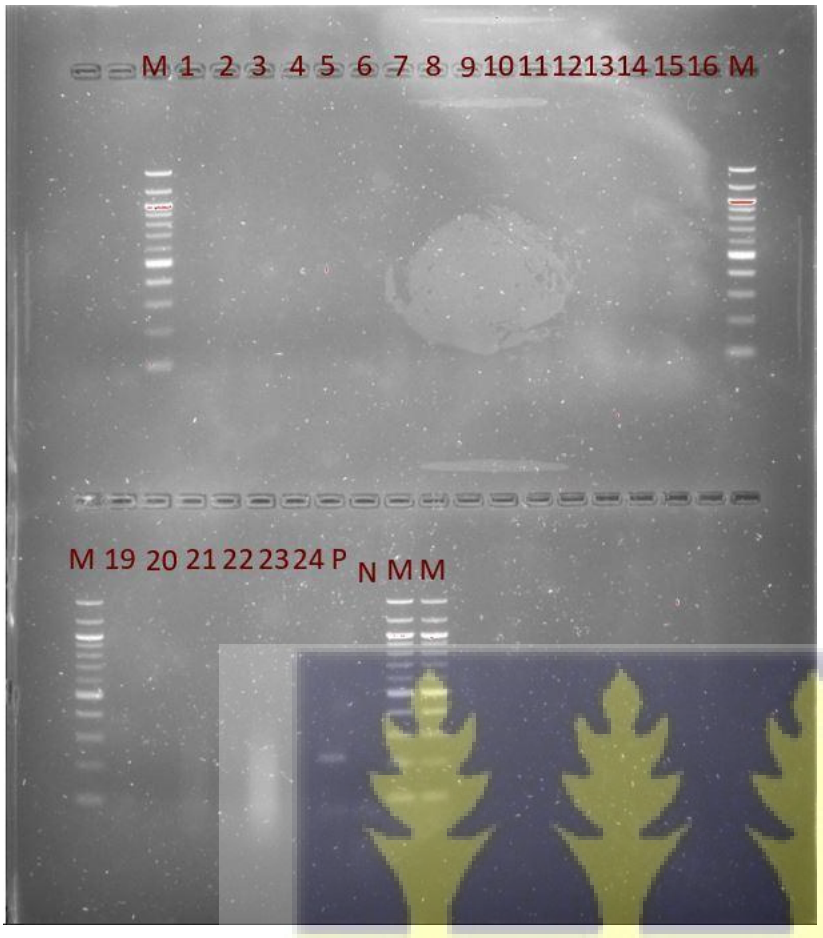

Figure 4: A gel image of the samples after the Pan-alphavirus PCR was run

4.5. Taxonomic Profile

From the total of 217 mosquito pools, 20 pools were randomly selected for sequencing. These comprised 10 pools from prospective collections and 10 pools from archived samples. The species distribution within these pools was as follows:

- 16 pools of *Culex quinquefasciatus/pipiens*
- 1 pool of *Culex antennatus*
- 1 pool of *Culex neavei*
- 1 pool of *Culex tritaeniorhynchus*
- 1 pool of *Culex zombaensis*

Following nucleic acid quantification using Qubit, 14 pools contained sufficient nucleic material for RT-PCR. A second Qubit measurement after RT-PCR confirmed that 10 pools were suitable for sequencing, all of which belonged to *Culex quinquefasciatus/pipiens*.

Figure 5 presents the taxonomic profile of microorganisms identified within these 10 pools, consisting of 9 from Accra and 1 from Navrongo. The taxonomic distribution revealed a diverse community of microorganisms associated with *Culex quinquefasciatus/pipiens,* as seen in Figure 5. The data highlight the predominance of Viridiplantae and Alveolata, with viruses representing a smaller but significant proportion of the microbial community associated with Culex mosquitoes.

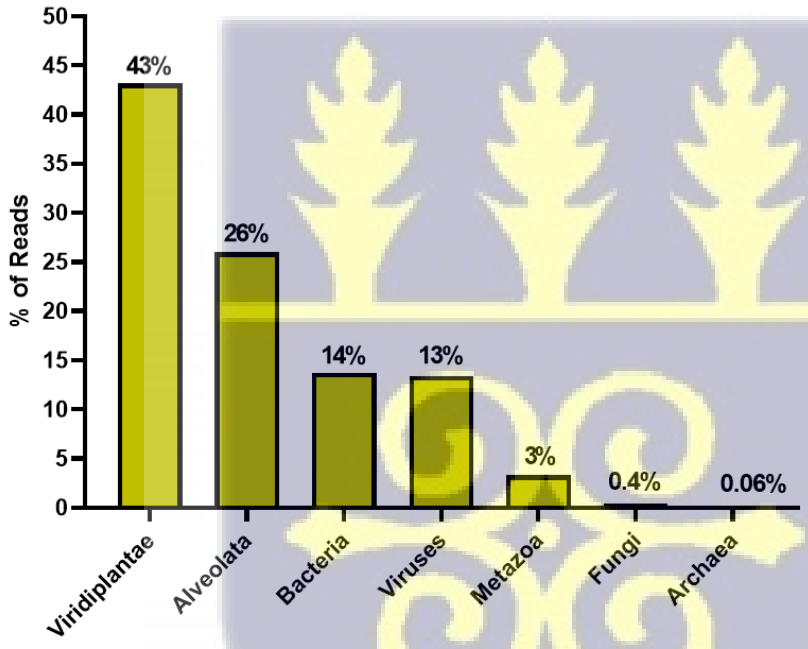

Figure 5: Taxonomic profile of Culex quinquefasciatus/pipiens pools in both sites

4.6. Viral Taxonomic Characterisation

As shown in Figure 6, the virome analysis revealed a diverse viral community comprising 18 distinct viral families, in addition to several unclassified viral groups. The viral composition was dominated by members of the Varidnaviria and Duplodnaviria realms. There was a large sequence for Caudoviricete, Flaviviridae, Orthoherpesviridae, and Retroviridae groups within the virus domain identified. This dataset includes various unclassified parts: 23% of ssRNA +strand viruses, 15% of Riboviria members, 12% of general RNA viruses (ShiM-2016), and smaller amounts of unclassified Retroviridae (8%) and Iflavirieridae (5%). Notable pathogenic families identified include Parvoviridae (5.7% abundance), Circoviridae (4.2%), and Solemoviridae (3.8%). RNA 40 (Navrongo) and EMB-P-1 did not show diversity in their viral profiling, whilst EMB-P-31 did not reveal any viral genome.

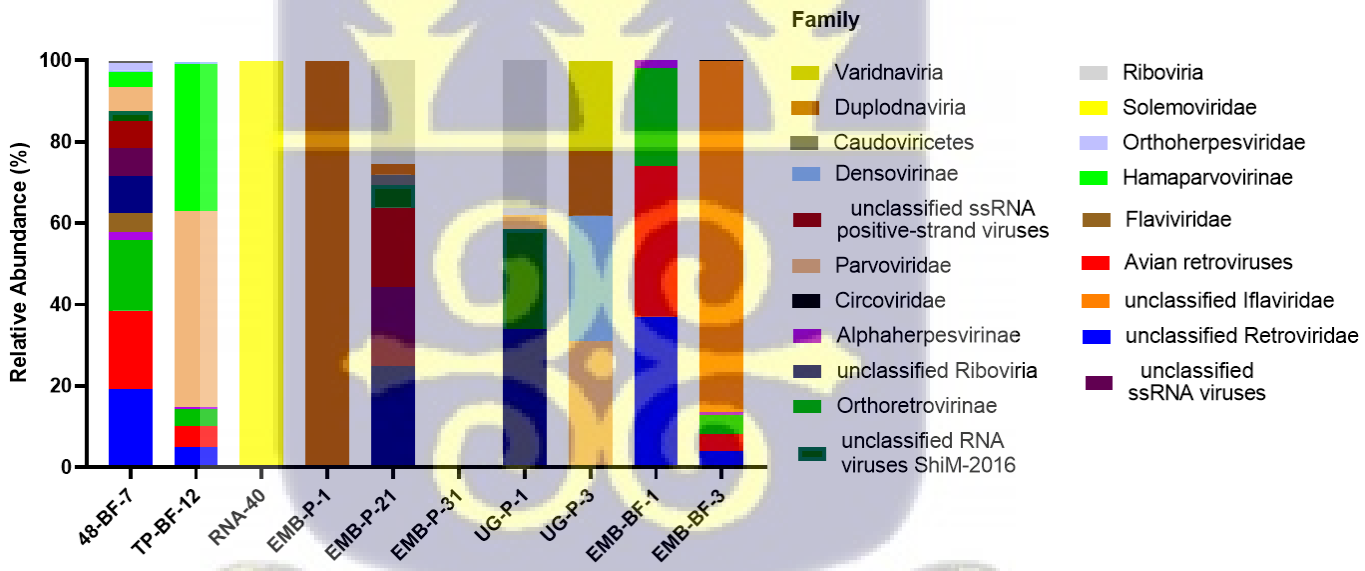

Figure 6: Taxonomic characterisation of the viral distribution for each sample

4.7. Viruses per Sample

Figure 7 shows the characterisation of the virome of *Culex pipiens/quinquefasciatus* mosquito pools. Twenty-seven (27) distinct viral species were identified, proving the vector's complex role as a reservoir for diverse viruses. The viral community found was dominated by Retroviridae,

especially avian-derived retroviruses (Avian leukosis/sarcoma viruses, Rous sarcoma virus). These viruses suggest frequent mosquito exposure to avian hosts. Unclassified RNA viruses represented by multiple strains (X1-X3) of novel influenza-like viruses and *Culex* flavivirus were also seen. Geographically segregated rhabdovirus variants (Grenada and Guadeloupe strains) and the Gallus gallus endogenous virus raise questions about host-virus co-evolution. Analysis of eight sample pools (48-BF-7, RNA 40, TP-BF-12, EMB series, and UG series) revealed consistent viral patterns.

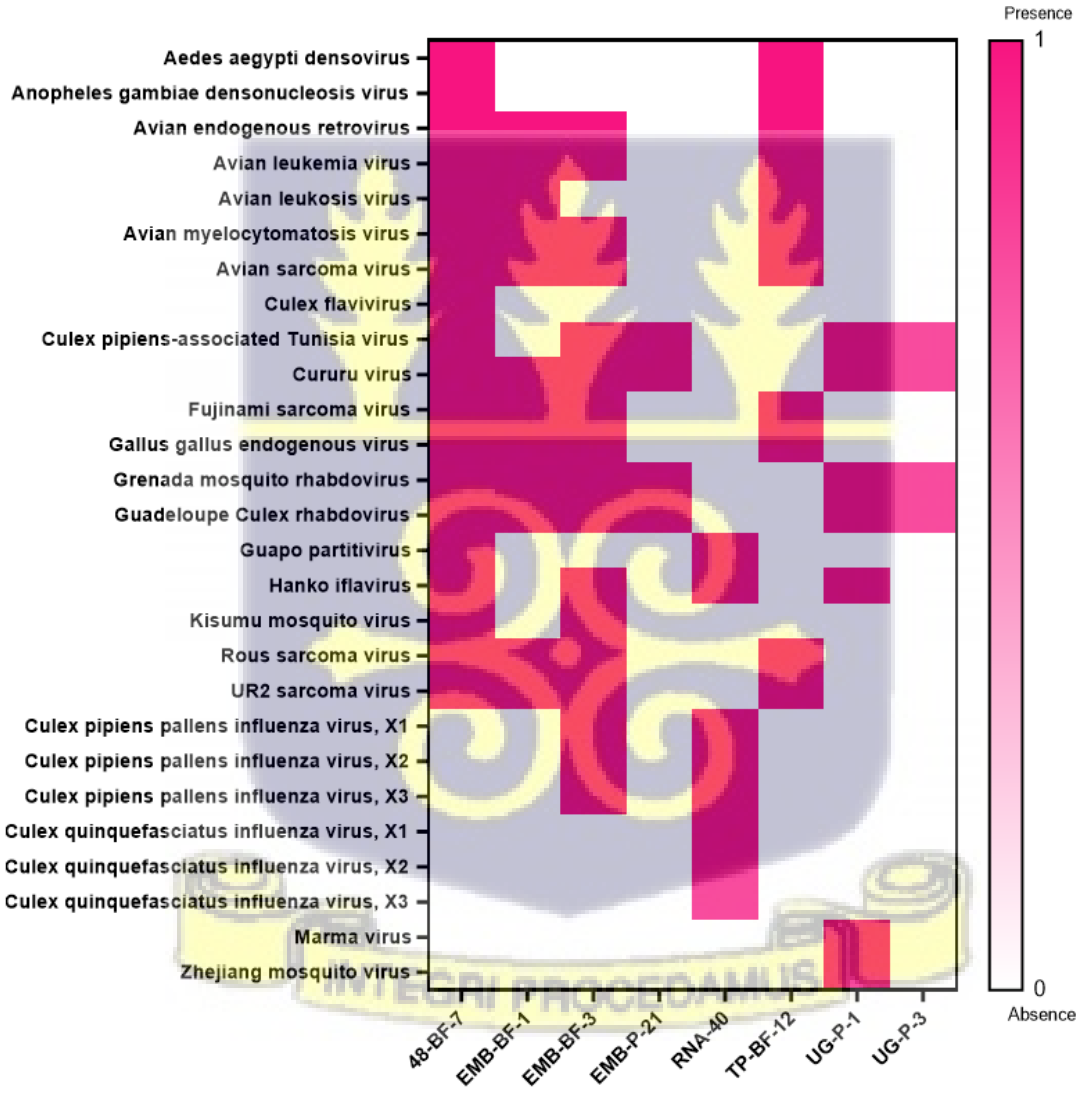

Figure 7: A heatmap showing the different viruses seen per sample

4.8. Genome Recovery

As shown in Figure 8, 60% of viral genomes recovered were found to be full genomes. Full genomes were described as genomes with 90% - 100% gene recovery. 40% of the genomes recovered were considered partial, as their gene recovery was below 90%. Most of the full-genome representation reflects successful sequencing depth and effective bioinformatics assembly.

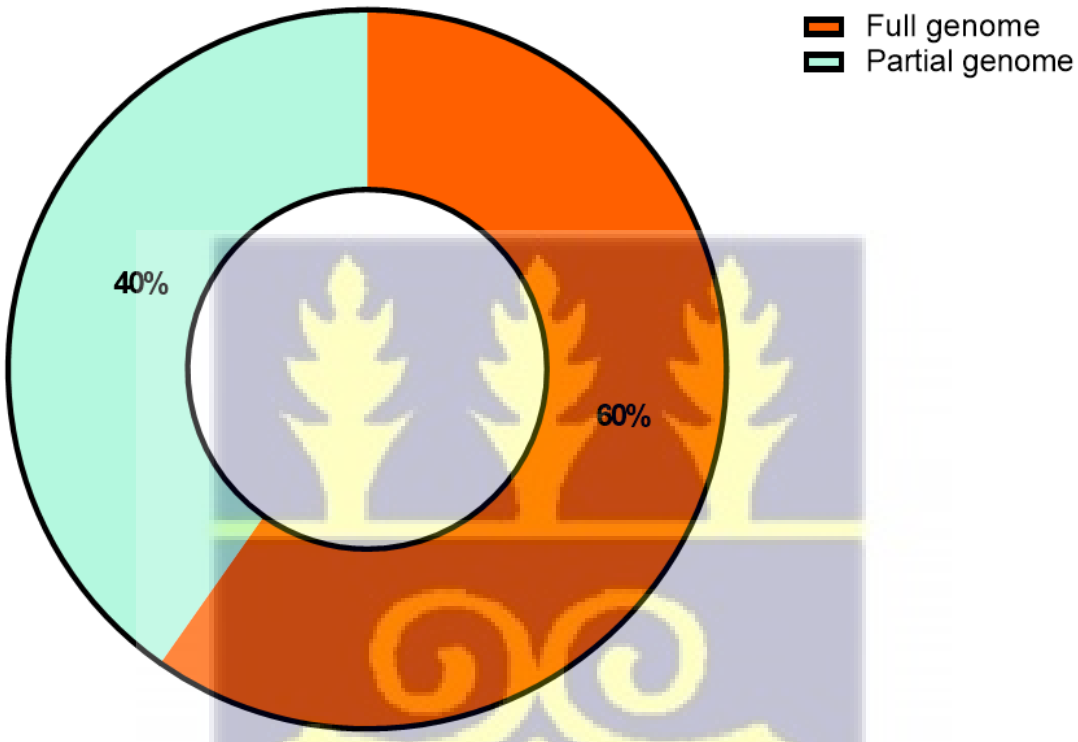

Figure 8: A doughnut chart of the type of genome recovered

4.9. Phylogenetic Analysis of Detected Viruses

Phylogenetic trees were constructed to elucidate the evolutionary relationships between viral sequences identified in this study and globally circulating strains. All trees were generated with 1,000 bootstrap replicates to assess node support, with values >70% indicating robust clustering.

4.9.1. *Aedes aegypti* densovirus

In Figure 9, the Ghana_2025 strain (48BF7) clusters within a distinct clade, showing evolutionary divergence from sequences isolated in the USA (2008 & 2018) and China (2004-

2016). This suggests potential local evolution of this densovirus lineage in Ghanaian mosquito populations.

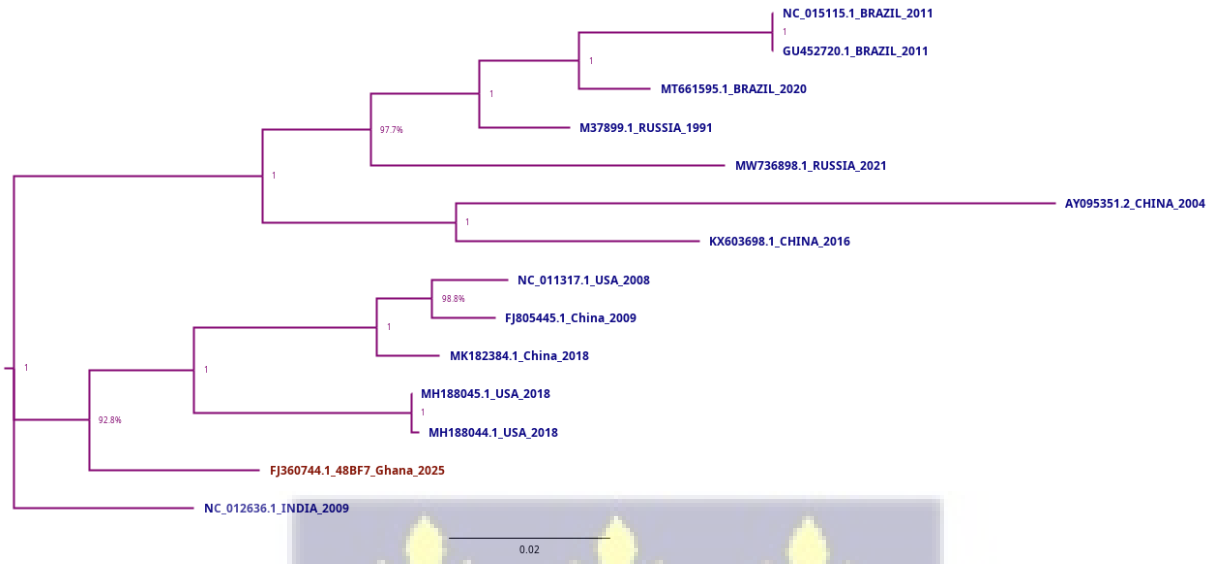

Figure 9: Phylogenetic tree of Aedes aegypti densovirus

4.9.2. *Anopheles gambiae* densonucleosis virus

Figure 10 shows that the Ghana_2025 strain (48BF7) forms a unique branch, separate from global sequences. While closely related to a 2009 Indian strain (NC_012636.1), it exhibits significant genetic distance from American and South American variants, showing possible geographic isolation.

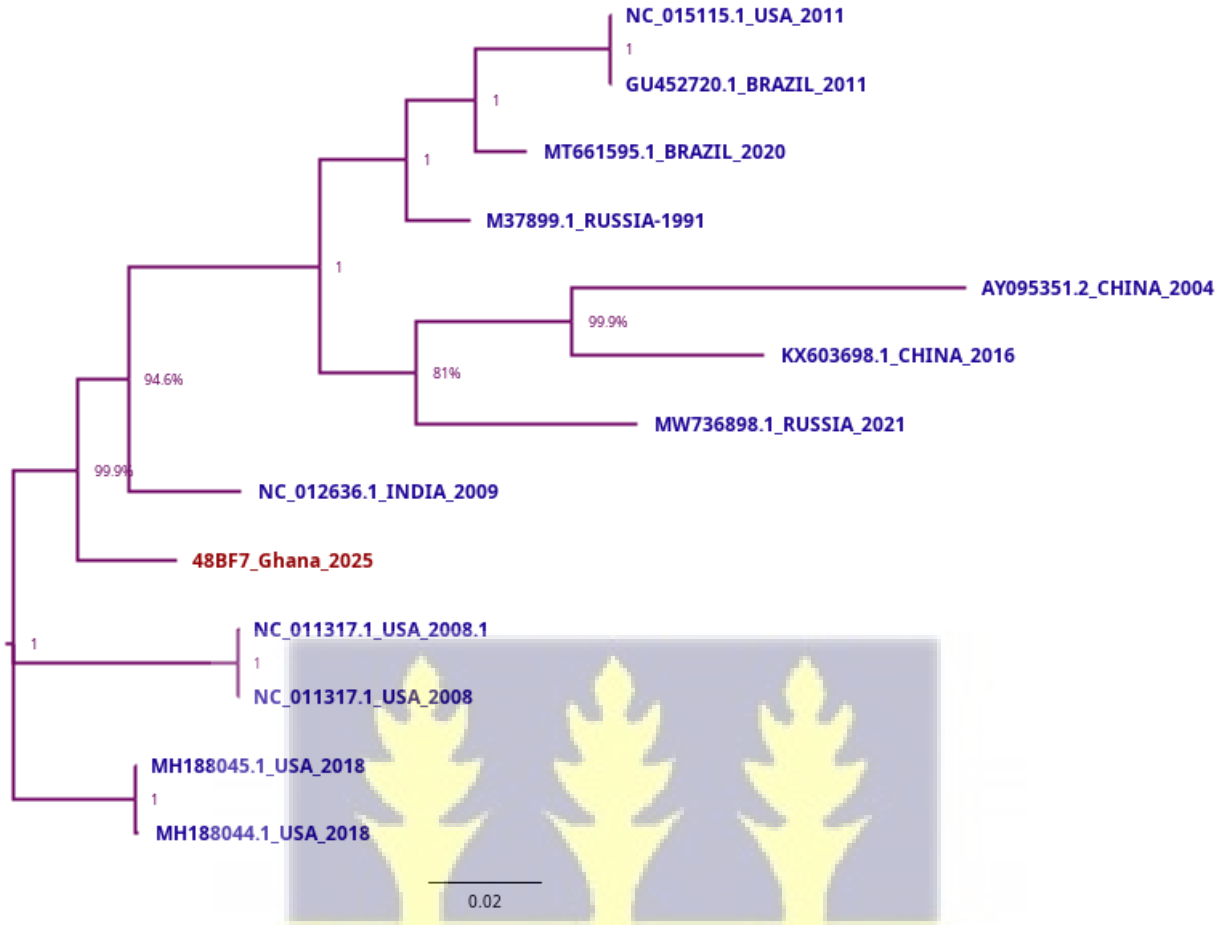

Figure 10: Phylogenetic tree of Anopheles gambiae densonucleosis virus

4.9.3. Avian leukosis virus

As seen in Figure 11, the Ghanaian sequences from 2025 (48BF7, EMBBF1, TPBF12) clustered together with a bootstrap support of 72.6%. This clade suggests a possible ancestral link to recent Indian (2022), with a lower bootstrap value of 50.5% supporting a likely cross-continental transmission pattern. Notably, the 48BF7 strain shares 72.6% similarity with the Indian variant.

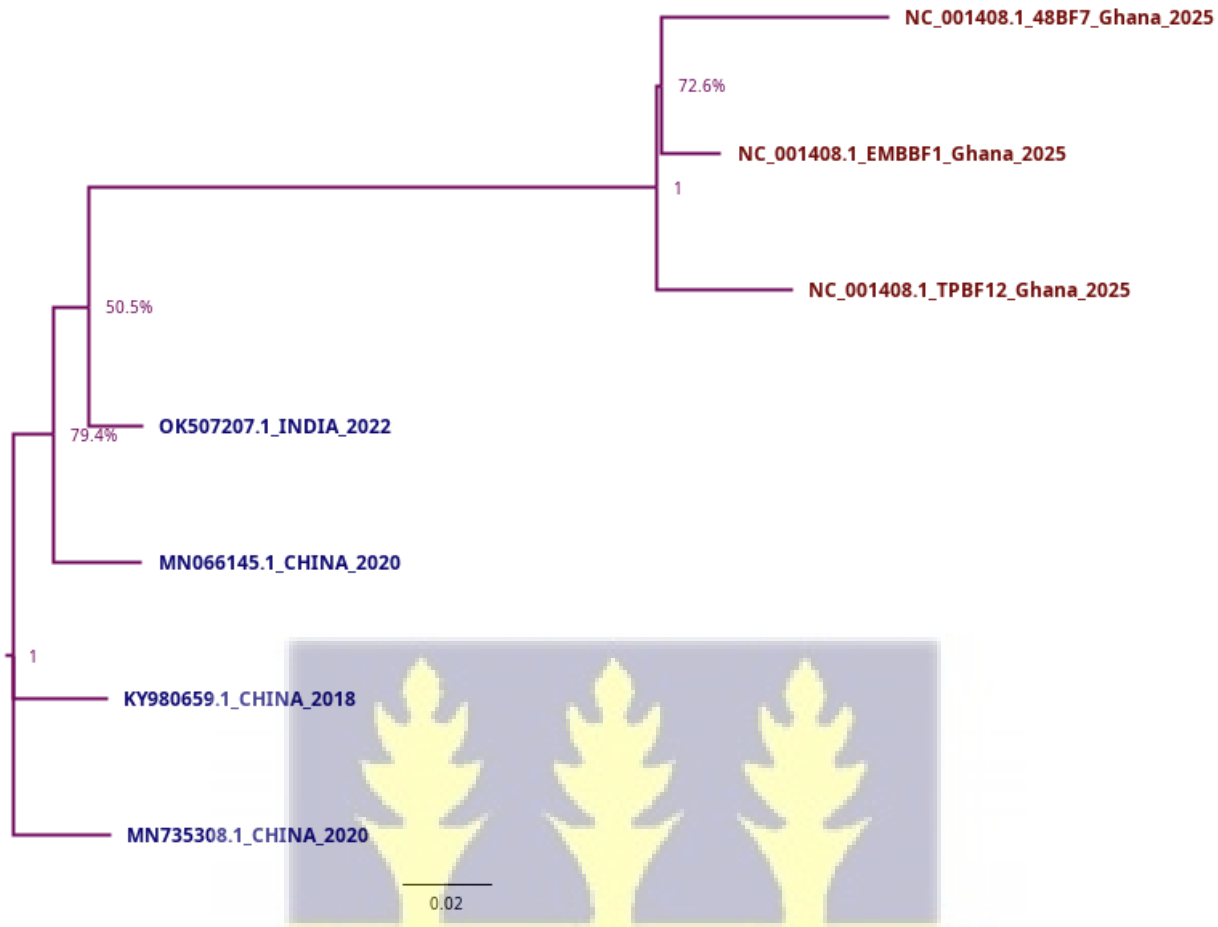

Figure 11: Phylogenetic tree of Avian leukosis virus

4.9.4. Avian myelocytomatosis virus

In Figure 12, two Ghana_2025 sequences (TPBF12, EMBBF1) formed a monophyletic group with 100% bootstrap support. This cluster shares a direct evolutionary lineage with a 1997 American strain (AF033809.1), suggesting historical transmission between North America and West Africa.

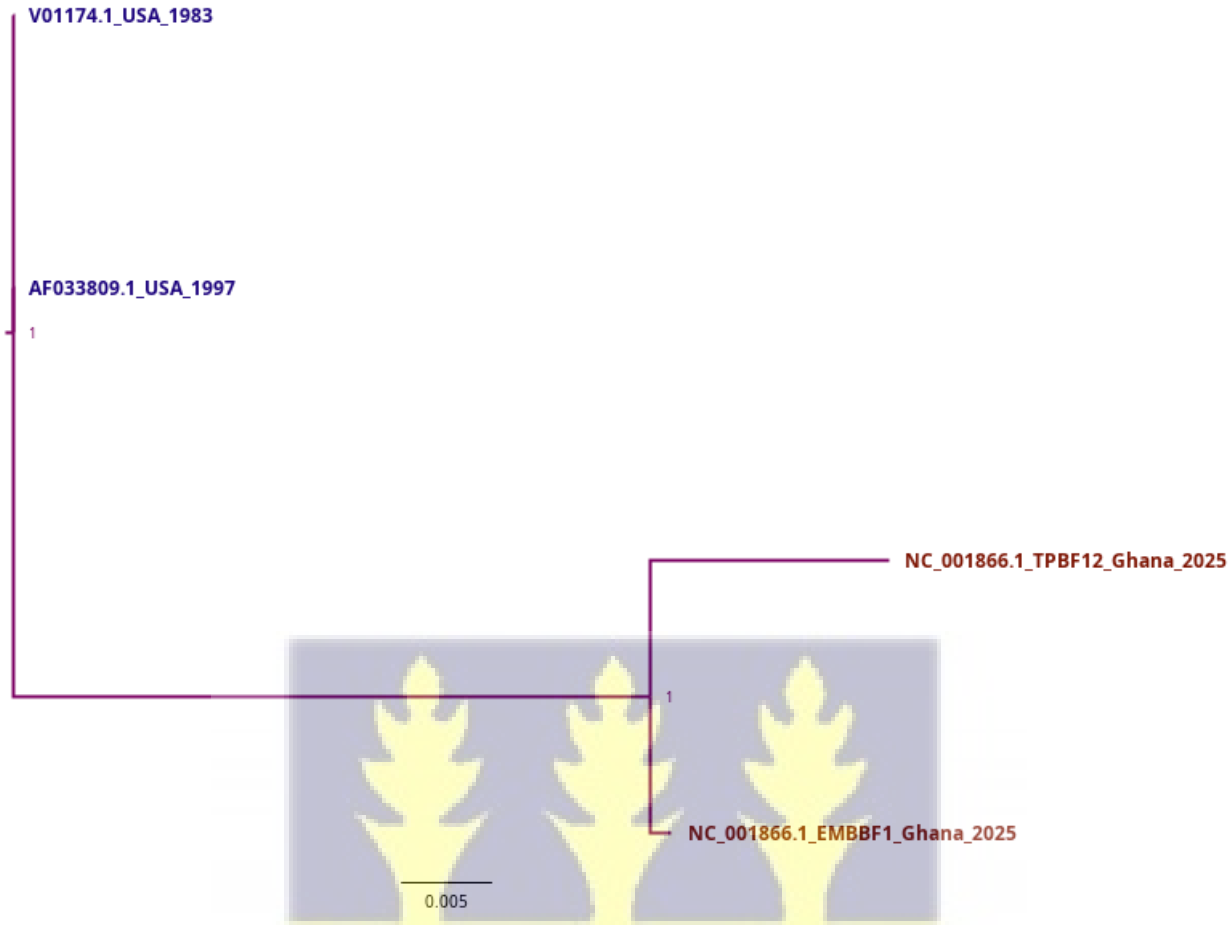

Figure 12: Phylogenetic tree of Avian myelocytomatosis virus

4.9.5. Avian sarcoma virus

As shown in Figure 13, the Ghana_2025 strain (TPBF12) occupies an intermediate phylogenetic position between contemporary Chinese variants (2014-2023) and historical American isolates (1980-2018). Its placement near a 2018 USA strain (NC_038922.1) suggests possible American-African viral exchange.

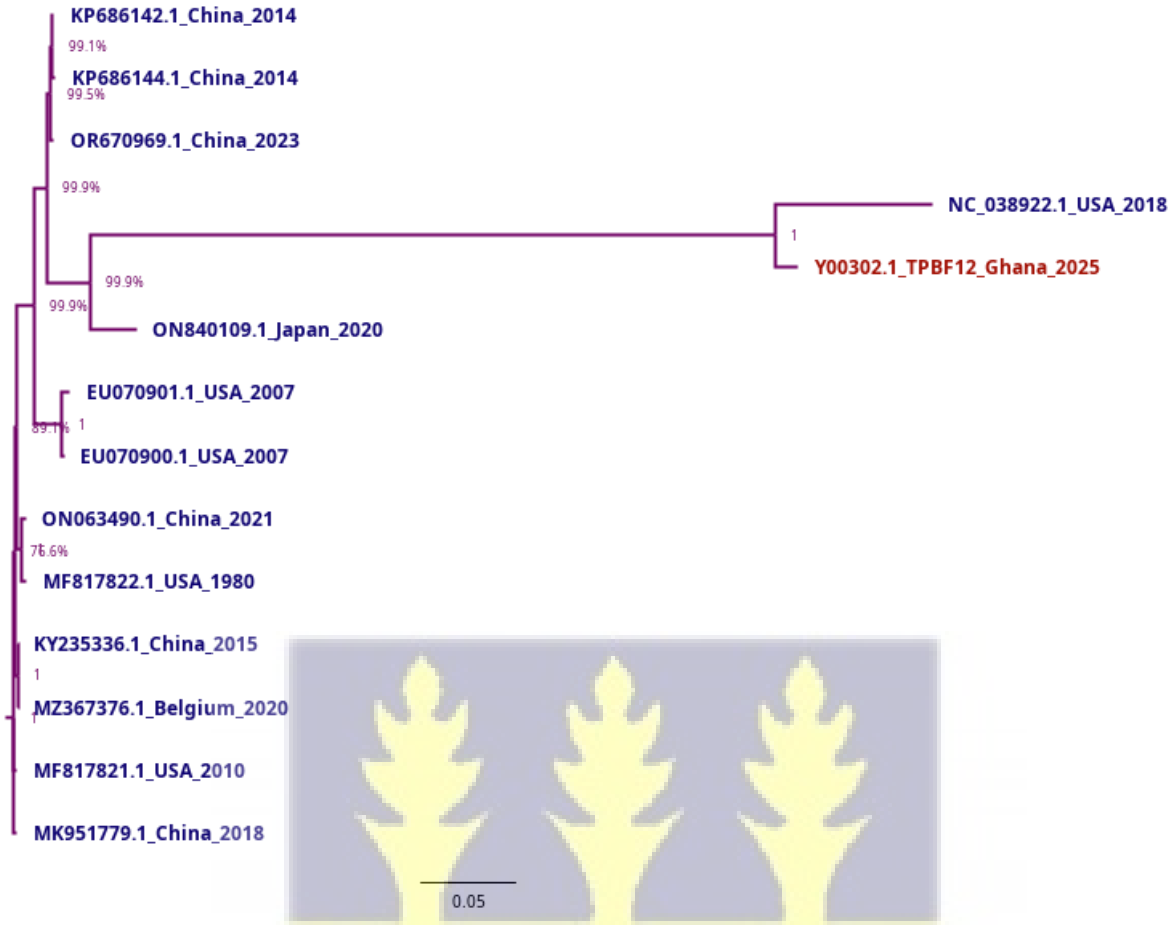

Figure 13: Phylogenetic tree of Avian sarcoma virus

4.9.6. Fujinami sarcoma virus

Figure 14 shows a phylogenetic tree of Fujinami sarcoma virus (FSV) sequences was constructed with 1000 bootstrap replicates to assess branch support. Sequences were labelled by accession number, country of origin, and year of isolation. The analysis revealed two well-supported clades: one with the Ghanaian sequences and another consisting of historical isolates from the USA (1982 and 2000). The Ghanaian isolates formed distinct clusters, with high bootstrap values (≥1.000), suggesting strong evolutionary divergence from the older USA strains. Branch length differences show varying degrees of genetic change, with the Ghana sequences showing relatively higher divergence from the inferred common ancestor.

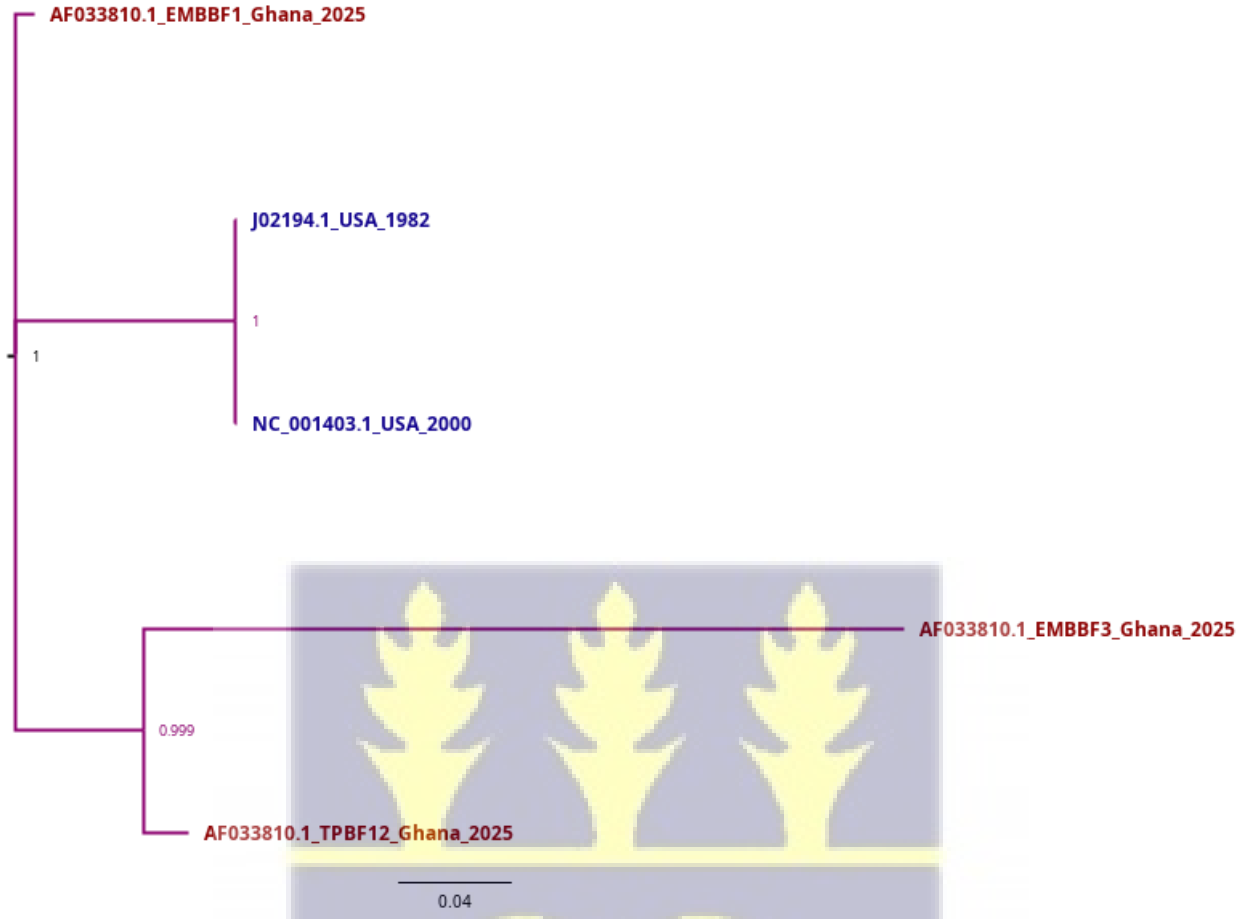

Figure 14: Phylogenetic tree of Fujinami sarcoma virus

CHAPTER FIVE

5.0. DISCUSSION

The preceding chapters have presented a multi-layered portrait of *Culex* mosquitoes circulating in the study area, integrating classical entomological indices with molecular and metagenomic read-outs. First, the entomological survey attained an unusually high taxonomic resolution for the region: 60.3 % of all mosquitoes recovered were identified to the *Culex pipiens/quinquefasciatus* complex, a proportion that sits at the upper end of published urban gradients in West Africa and thereby sharpens the evidentiary base for risk mapping. Second, the blood-meal analysis retained 27 % blood-fed females, an appreciable yield given that collections were larva-derived and therefore biased toward non-blood-fed adults. Third, the conventional PCR pipeline delivered consistently negative arbovirus screens, thus strengthening the argument for true absence. Finally, the metagenomic libraries recovered one of the most deeply sequenced *Culex* viromes yet reported from sub-Saharan Africa (18 recognised families plus 40 % unclassified viral signal). These data give real-world evidence that wide-range sequencing can detect viruses that PCR screens may skip.

5.1. Culex Distribution

It is worth noting that *Culex* mosquitoes are distributed globally, serving as vectors for several crucial diseases. The abundant species are usually *Culex pipiens* and *Culex quinquefasciatus* in both urban and peri-urban environments in Africa, Asia, and the Americas, often outnumbering their other relatives. This is generally due to *Culex*'s high adaptability to human habitats and polluted water sources (Amao et al., 2018; Bashar et al., 2016; González et al., 2021; Martinho et al., 2024; Nchoutpouen et al., 2019; Shehata et al., 2022). The dominance of *Culex pipiens/quinquefasciatus* is often linked to their ability to adapt to urban habitats and polluted

water, while rarer species may be more sensitive to habitat changes or have more specialised breeding requirements (Bashar et al., 2016; Gorris et al., 2021; Nchoutpouen et al., 2019; Shehata et al., 2022; Yan et al., 2024). The abundance of *Culex pipiens/quinquefasciatus* is alarming since literature suggests they transmit arboviruses like West Nile Virus, St. Louis encephalitis, and Japanese encephalitis virus (Gould et al., 2025). In other countries such as Nigeria, Cameroon, Bangladesh, and Spain, the proportion of *Culex pipiens* and *Culex quinquefasciatus* from different mosquito species trapped was shown to be 49.4%, 79.4%, 21.7%, and 45.1%, respectively, being the highest proportion of different mosquito species recovered (Amao et al., 2018; Bashar et al., 2016; González et al., 2021; Nchoutpouen et al., 2019), and in Egypt, it was shown to be the highest of the mosquito species retrieved after surveillance (Shehata et al., 2022).

5.2. Blood Feeding Distribution

It was observed that 73% of the mosquitoes collected were non-blood-fed and 27% were blood-fed. In this study, males were not accounted for in these statistics. Among the female population, a 27% proportion of blood-fed mosquitoes was observed from the collected data. This low prevalence could have resulted from using non-blood-fed mosquitoes recovered from larvae that had matured to adults. Again, blood-feeding rates are not fixed and can vary with habitat, host abundance, mosquito species, and even interventions such as insecticide-treated nets, which might reduce feeding success (Barreaux et al., 2023; Stephenson et al., 2019; Wu et al., 2023). *Culex* spp. are primarily bird feeders (ornithophilic) and feed on humans in the absence of their preferred host. The site of mosquito collection had fewer birds, which could account for the reduced percentage of blood-fed mosquitoes. Also, these areas use mosquito nets, which may have reduced their success rate of obtaining a blood meal. This aligns with the established population of mosquitoes across multiple countries, having a 60% - 90% non-blood-fed mosquito prevalence in mosquito

catches and a 10% - 40% blood-fed mosquito prevalence (Brugman et al., 2017; Melgarejo-Colmenares et al., 2022; Stephenson et al., 2019; Wu et al., 2023).

5.3. Conventional PCR Results

Several *Culex* mosquito surveillance studies have reported the presence of both flaviviruses and alphaviruses, yet this study found no evidence of these viruses in circulation. The absence of these viral families from PCR could indicate that the viruses were not in circulation at the site of collection during the mosquito collection, or due to the long storage in the -80°C, the RNA was degraded and could show false negatives. This calls for continuous surveillance. The body of work done shows that findings can vary by geography, time and mosquito species recovered. For example, a study in Qom, Iran, which collected over 83,000 mosquitoes (including *Culex* species), found no evidence of flavivirus or alphavirus infection using genus-specific RT-PCR assays, which agrees with the negative results described in the initial gel electrophoresis images 4 & 5 (Abedi-Astaneh et al., 2024).

Similarly, in western Kenya, RT-PCR screening of mosquito pools (including *Culex*) found no alphavirus RNA; however, some pools were positive for flavivirus, although insect-specific (Lwande et al., 2024). In contrast, surveillance studies in the Colombian Caribbean identified several arboviruses, including West Nile virus, St. Louis encephalitis virus, and *Culex* flavivirus (Hoyos-López et al., 2016; Miranda et al., 2019). This shows active circulation of these viruses within local populations. Additionally, on the Yucatán Peninsula, Mexico, a high prevalence of *Culex* flavivirus and a novel flavivirus was found in *Culex quinquefasciatus*, although no human-pathogenic flaviviruses were detected (Farfan-Ale et al., 2009).

5.4. Taxonomic Diversity of Microorganisms in Mosquito Samples

Whole-body metagenomic sequencing captures a broader range of taxa (including environmental and dietary DNA) compared to gut-specific studies, which often report higher bacterial proportions (Sadeghi et al., 2018a; Wang et al., 2021). The metagenomic data from the pooled *Culex* mosquitoes show a complex and a diverse microbial community, with Figure 5 showing Viridiplantae (43%) and Alveolata (26%) as the most prevalent taxa and lower proportions of Bacteria (14%), Viruses (13%), Metazoa (3%), Fungi (0.4%), and Archaea (0.06%). Viridiplantae, representing plants, were abundant, and plant-derived sequences likely reflect the mosquitoes' frequent contact with plant material, such as nectar feeding or environmental exposure. This is a common finding in metagenomic studies of whole mosquitoes, where environmental DNA can be co-extracted with microbial DNA (Guégan et al., 2018; Sadeghi et al., 2018a; Zhang et al., 2021). The Alveolata group includes protozoan symbionts and parasites, such as gregarines, which are often found in mosquito microbiomes and can be highly abundant (Sadeghi et al., 2018a; Zhang et al., 2021). Metagenomic studies have identified numerous novel viral genomes, but their abundance is typically lower than that of plant and protozoan DNA, and it is borne out in this study with a 13% viral recovery. Fungi and Archaea are usually present at very low levels, consistent with previous findings that fungi and archaea are minor components of the mosquito microbiome (Chandler et al., 2015; Zhang et al., 2021).

5.5. Viral Taxonomic Profile within Each Mosquito

The metagenomic analysis uncovers a highly diverse virome, comprising 18 viral families and a substantial proportion of unclassified viral groups, primarily dominated by Varidnaviria and Duplodnaviria. This pattern of extensive diversity and a large fraction of unclassified or novel viruses is usually seen in modern virome studies. The large number of unclassified or novel viruses shows the genuine complexity of viral communities and the current limitations of

reference databases and classification systems (Wommack et al., 2012a; Simmonds et al., 2017a; Wolf et al., 2020; Zolfo et al., 2024a). The presence of both DNA and RNA virus families, as well as a large proportion of unclassified ssRNA viruses and Riboviria, highlights the variety of viral types captured by metagenomic sequencing (Dolja & Koonin, 2018; Wolf et al., 2020; Zolfo et al., 2024b). The detection of pathogenic families such as Parvoviridae, Circoviridae, and Solemoviridae is important, as these groups are often associated with disease in various hosts and are frequently targeted in virome surveillance (Cebriá-Mendoza et al., 2021; Lu et al., 2025; Shi et al., 2019b). The dominance of Orthoherpesviridae and Retroviridae is consistent with findings from diverse environments, such as human blood and vaginas, mosquitoes, or aquatic habitats, where Retroviridae and Flaviviridae are common among eukaryotic viruses (Cebriá-Mendoza et al., 2021; Lu et al., 2025; Shi et al., 2019b; Wolf et al., 2020). A significant portion of this dataset was unclassified (e.g., 23% ssRNA +strand, 15% Riboviria, 12% general RNA viruses), which tends to be the case in virome studies due to what in literature is termed as "viral dark matter," which are sequences with no close matches in current databases (Eric Wommack et al., 2012b; Wolf et al., 2020; Zolfo et al., 2024c). This underscores the ongoing need for expanded reference databases and improved bioinformatics tools to better resolve viral taxonomy (Rampelli et al., 2016; Roux et al., 2017; Simmonds et al., 2017b). The lack of viral diversity in RNA 40 and EMB-P-1, and the absence of viral genomes in EMB-P-31, may reflect biological variation, sample quality, or technical limitations such as sequencing depth or extraction efficiency. Such variability is common, highlighting the importance of technical replicates and careful interpretation of negative results (Roux et al., 2017; Shi et al., 2019b; Sutton et al., 2019). From studies conducted on virome analysis, it is expected to find high diversity and a substantial number of unclassified viral groups. Metagenomic studies consistently show that *Culex*

pipiens/quinquefasciatus mosquitoes have a wide range of viral species. Some studies have identified over 30 virus families spanning vertebrate, invertebrate, plant, and unclassified viruses (Atoni et al., 2018; Sadeghi et al., 2018b). The virome composition is influenced by factors such as geography, mosquito diet, and environmental exposure. Avian-derived retroviruses are often detected, and this aligns with findings from this study, supporting the idea that these mosquitoes often feed on avian hosts and may aid cross-species viral transmission (Atoni et al., 2018; Sadeghi et al., 2018b). Unclassified RNA viruses, such as novel influenza-like viruses, have been seen, highlighting the ongoing discovery of previously unknown viral strains in mosquito populations. The detection of geographically segregated rhabdovirus variants like Grenada mosquito rhabdovirus and Guadeloupe Culex rhabdovirus strains and endogenous avian viruses, e.g., Gallus gallus endogenous virus, suggests dynamic host-virus coevolution and possible local adaptation (Atoni et al., 2018; Sadeghi et al., 2018b; Shi et al., 2019a).

5.6. Phylogenetic Clustering, Local Evolution, Cross-Continental Transmission and Ancestral Linkages

The distinct clustering of the Ghana_2025 *Aedes aegypti* densovirus and *Anopheles gambiae* densonucleosis virus strains, separate from global reference sequences, suggests local evolution and possible geographic isolation (Drummond et al., 2003; Pybus & Rambaut, 2009; Strauss & Strauss, 2014). Such patterns tend to reflect rapid mutation rates, host adaptation, and limited gene flow between regions. The observed >5% genetic divergence from American and South American variants further supports the idea of region-specific viral lineages, a phenomenon frequently documented in both RNA and DNA viruses (Forni et al., 2017; Pybus & Rambaut, 2009). The clustering of Ghanaian avian leukosis and myelocytomatosis virus sequences with Indian, Chinese, and American strains, supported by strong bootstrap values, might indicate a

historical or recent cross-continental transmission event. This tracks with the global movement of viruses via hosts or vectors and the network-like nature of viral evolution, where both vertical descent and horizontal exchanges, i.e., recombination and reassortment, shape phylogenetic relationships (Chan et al., 2013; Koonin et al., 2015; Nasir & Caetano-Anollés, 2015). The ancestral linkage and intermediate phylogenetic positions observed in the data have a similar pattern to that seen in other studies of avian and mosquito-borne viruses (Boni et al., 2020; Pybus & Rambaut, 2009).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

This study provides a characterisation of *Culex* mosquito populations from two ecologically distinct urban and peri-urban environments in Ghana, Accra and Navrongo, using high-throughput Illumina HiSeq sequencing. The findings reveal a complex and diverse array of viruses spanning multiple families, including a substantial representation of avian-associated retroviruses. The detected viral diversity reflects not only the mosquitoes' host-feeding patterns but also broader ecological interactions within tropical urban landscapes. Phylogenetic analyses revealed geographically structured clustering of Ghanaian avian leukosis and myelocytomatosis virus sequences, grouping closely with Indian, Chinese, and American strains with strong bootstrap support. Such patterns might suggest possible historical introductions or ongoing cross-continental viral exchange, potentially mediated by migratory birds, international poultry trade, or other anthropogenic drivers. The presence of intermediate phylogenetic positions and ancestral linkages potentially indicates that vertical transmission, recombination, and reassortment are active forces shaping the evolutionary trajectories of these viruses. Although classical human-pathogenic arboviruses were not detected at significant levels, the documented richness of mosquito-associated viruses underscores the underappreciated role of *Culex* mosquitoes as reservoirs or vectors for a broader virome than traditionally recognised. Collectively, these results provide a critical molecular baseline for *Culex* vector viromes in West Africa, highlight the transboundary nature of avian viral ecology, and emphasise the necessity for continuous, targeted surveillance and strengthened biosecurity frameworks to mitigate the risk of emergent or re-emergent arboviral diseases in urban settings.

6.2. Limitations

1. The use of pooled mosquito samples (ranging from 2 to 20 individuals) may mask individual infection variability and influence viral detection sensitivity. Furthermore, pool size differences between sites could confound direct comparisons of viral prevalence.
2. Detection of viral sequences via metagenomics does not inherently indicate active infection or transmissibility.
3. Without screening for endosymbionts or viral replication markers such as subgenomic RNAs, this study cannot assess the influence of symbionts on the virome or confirm viral replication status within mosquitoes.
4. The cross-sectional design focusing on two urban sites captures only a temporal snapshot, limiting insights into seasonal or broader regional virome dynamics.
5. The study relies solely on sequence-based evidence without confirmatory culturing or infectivity assays, limiting the ability to fully ascertain the biological relevance and potential pathogenicity of the detected viruses.
6. The use of archived mosquitoes may produce false negatives due to the degradation of viral RNA over time.

6.3. Recommendations

1. Future research should couple metagenomic viral profiling with entomological data, blood meal analysis, and ecological studies to more precisely unravel vector-host-virus relationships, transmission dynamics, and vector competence in urban ecosystems.
2. Given endosymbionts such as *Wolbachia*'s known modulatory effects on mosquito viromes and pathogen transmission, incorporating molecular screening for endosymbiotic

bacteria is recommended. This would elucidate interactions that may influence viral replication, prevalence, and vectorial capacity.

3. To capture how mosquito viruses change over time and vary between places, longitudinal surveillance across multiple seasons and an extended range of geographical sites should be included. This would help distinguish stable from transient virome components and better infer epidemic potential.
4. Complementary laboratory studies, including virus isolation, infectivity assays, and transcriptomic analyses, are necessary to confirm active viral replication within mosquito hosts, differentiating persistent infections from blood meal-derived viral remnants.
5. Expanding investigations to include different mosquito genera such as *Aedes* and *Anopheles,* within the same geographic locations will provide a broader ecological and epidemiological context, assisting in disentangling genus-specific virome features and zoonotic risk profiles.

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