UNIVERSIDADE NOVA
DE LISBOA

VECTOR COMPETENCE OF *AEDES AEGYPTI* FROM SÃO TOMÉ AND PRÍNCIPE FOR THE TRANSMISSION OF WEST NILE VIRUS

Pedro Rafael Martins e Marmé

A dissertation submitted in fulfillment of the requirements for the Degree of Master in Medical Microbiology

Master in association: Faculdade de Ciências Médicas | NOVA Medical School; Instituto de Higiene e Medicina Tropical; Instituto de Tecnologia Química e Biológica António Xavier; NOVA School of Science & Technology

October, 2024

UNIVERSIDADE NOVA
DE LISBOA

VECTOR COMPETENCE OF *AEDES AEGYPTI* FROM SÃO TOMÉ AND PRÍNCIPE FOR THE TRANSMISSION OF WEST NILE VIRUS

Pedro Rafael Martins e Marmé
Supervisors: Gonçalo Seixas, Junior Researcher at Instituto de Higiene e Medicina Tropical,
Ricardo Parreira, Associate Professor at Instituto de Higiene e Medicina Tropical and
Carla A. Sousa, Associate Professor at Instituto de Higiene e Medicina Tropical

A dissertation submitted in fulfillment of the requirements for the Degree of
Master in Medical Microbiology

Master in association: Faculdade de Ciências Médicas | NOVA Medical School; Instituto de Higiene e Medicina Tropical; Instituto de Tecnologia Química e Biológica António Xavier; NOVA School of Science & Technology

nms.unl.pt

October, 2024

Peer-reviewed published manuscript directly related to the work presented in this thesis:

Marmé, R., Tomaz, F., Sousa, C. A., Pinto, J., Lanzaro, G. C., Parreira, R., & Seixas, G. (2024). Vector competence of *Aedes aegypti* from São Tomé and Príncipe for West Nile virus transmission. Microorganisms, 12(10), 2038. https://doi.org/10.3390/microorganisms12102038

Acknowledgements

-Ao Prof Dr. Gonçalo Seixas meu orientador, um farol, um amigo que me apoiou, de maneira magnânima, que me ajudou a ultrapassar as montanhosas dificuldades que foram surgindo e que com o seu apoio e ensinamentos se transformavam em pequenas colinas. O seu incentivo impulsionou-me em todas as situações, mesmo nas mais críticas e difíceis, ajudou-me a seguir em frente, cada conversa me fazia sentir mais forte e capaz. Susteve-me em cada dúvida e hesitação, mostrou-me diligentemente uma promessa de futuro deveras interessante, mesmo quando teve necessidade de me corrigir nunca me desmotivou, mas proporcionou-me a possibilidade de desenvolver e melhorar esta investigação. Um eterno agradecimento pela confiança e pelos conhecimentos transmitidos e adquiridos que sem a sua ajuda não teriam sido possíveis;

-Ao Prof. Dr Ricardo Parreira obrigado pelo seu apoio, disponibilidade e conselhos assertivos que permitiram ultrapassar fases complicadas minorando-as e auxiliando no impulso deste projeto;

-À Prof Drª Carla Sousa agradeço pela disponibilidade e estímulo constante e confiança em etapas basilares deste percurso;

-Ao Prof Dr Paulo Almeida um imenso obrigado pelo seu constante incentivo, pelo crédito e apoio permanentes que me concedeu e que permitiram a realização e encaminhamento deste projeto. O seu incentivo permitiu a descoberta de um futuro que com a sua ajuda se tornou claro e realmente interessante;

-À Profª Dra Teresa Novo obrigado pela sua paciência e orientação num início difícil mas que com o seu auxílio se tornou possível e estimulante;

-À Rita que amavelmente cedeu o seu parco tempo para se disponibilizar e ajudar no desenvolvimento deste projeto o meu muito obrigado;

-Ao Filipe (futuro deutsch) a minha gratidão, um amigo que o IHMT me concedeu, que me inspirou e me transmitiu conhecimentos, tantas vezes preterindo o seu trabalho para me ajudar e em que nem mesmo a distância foi impeditiva da sua ajuda em toda e qualquer dúvida, sempre disponível mesmo para as mais insignificantes;

-Ao Prof Dr Manuel Tomé, uma das primeiras pessoas a mostrar-me que eu seria capaz de avançar nos meus conhecimentos e de superar desafios. Pelas palavras encorajadoras que conduziam a positivos e importantes desfechos. Um enorme obrigado pelo seu constante incentivo e confiança;

-Aos meus amigos que me vão mandando mensagem a perguntar como estão as coisas e se estou bem;

-Aos meus avós, em especial ao meu avô José Portugal que tantas vezes deixou de lado os seus afazeres para me ir buscar, para me ajudar e cuidar de mim, por me ter ensinado tantas coisas úteis e por ter pautado os seus dias pelas minhas necessidades tendo-lhe dado sempre primazia… um eterno obrigado;

-Aos meus padrinhos e prima que me acolheram como uns segundos pais, que reorientaram toda a sua vida para a emparceirar com a minha, para me cuidar, pelo seu apoio, suporte e tanta mas tanta ajuda. Pelo carinho, força e incentivo para resolver os meus problemas e conflitos tantas vezes em detrimento da sua vida …um imenso obrigado e agradecimento infinito e eterno;

-À Susana por me ter dado o seu carinho, por ter sido uma boa ouvinte, uma boa amiga e uma pessoa em quem eu pude confiar e desabafar nesta fase tão difícil e turbulenta que foi a escrita que também contou com a sua preciosa ajuda e em tão pouco tempo (relativamente) ter tido um impacto positivo tão significativo na minha vida;

-E por último, mas não menos importante: Aos meus pais por me terem apoiado em todas as fases do meu percurso, que sempre deixaram a minha vida o mais fácil e sem problemas possível, tendo para isso sacrificado horas de sono, horários de almoço e jantar, tantos quilómetros fizeram tendo de trabalhar no dia a seguir; independentemente dos erros que cometia tinha sempre o seu apoio e mostravam sempre uma forma de aprender com as falhas e crescer como

ser humano. Por serem uma inspiração da pessoa que eu quero ser, resilientes, fortes, desenrascados e carinhosos!

Abstract

Vector-borne diseases are a growing global health concern, particularly in tropical and subtropical regions where climatic conditions favor the proliferation of vectors such as mosquitoes. Among the most prominent vector species, *Aedes aegypti* plays a central role in transmitting significant arboviral diseases, including dengue, Zika, chikungunya, and yellow fever. Although *Ae. aegypti* is well-known for transmitting these viruses, its potential role in transmitting other arboviruses, such as West Nile Virus (WNV), is less understood. WNV is a neuroinvasive virus primarily transmitted by mosquitoes of the *Culex* genus, which circulate the virus between birds, its primary hosts, and mosquitoes. However, globalization, increased human mobility, and migratory birds pose the risk of WNV introduction into previously unaffected regions, such as São Tomé and Príncipe (STP). This study aims to address a critical gap in the literature by investigating the vector competence of *Ae. aegypti* mosquitoes from STP for WNV transmission. Although no WNV cases have been documented in STP to date, the region's strategic location along migratory bird routes highlight the potential for WNV introduction via infected birds. Evaluating the vector competence of *Ae. aegypti* in this context is essential for assessing future risks and implementing preventive measures.

In this study, laboratory-based experiments were conducted to assess the ability of local *Ae. aegypti* mosquitoes to acquire, maintain, and transmit WNV. Key parameters such as infection, dissemination, and transmission rates were evaluated at multiple time points (7, 14, and 21 days post-infection, dpi). The findings reveal that, *Ae. aegypti* from STP can become infected with WNV, but the transmission potential remains relatively low, infection rates increased progressively from 5% at 7 dpi to 35% at 21 dpi, suggesting that although these mosquitoes can acquire the virus, their susceptibility may be limited compared to more efficient WNV vectors like *Culex* species. Viral dissemination, representing the spread of the virus from the mosquito's midgut to other tissues such as the salivary glands, peaked at 100% by 14 dpi, but subsequently declined to 43% at 21 dpi. This decline suggests that while the virus can successfully replicate and spread within the mosquitoes initially, factors such as immune responses or resource depletion may limit further dissemination over time. More critically, the ability of the mosquitoes to transmit the virus—measured as transmission rate and efficiency—remained low throughout the study, with only a small proportion of mosquitoes effectively transmitting WNV to new hosts. This low transmission potential aligns with previous research suggesting that *Ae. aegypti* is a less competent vector for WNV compared to *Culex* species, which are the primary vectors for the virus.

Additionally, experiments were performed on the laboratory-reared Rockefeller strain of *Ae. aegypti*, a strain widely used in research. Notably, none of the mosquitoes from this strain showed signs of WNV infection, dissemination, or transmission. This finding likely reflects either inherent resistance to WNV infection or the impact of genetic drift and inbreeding due to long-term laboratory rearing, which may reduce vector competence. Such findings underscore the challenges of extrapolating laboratory-based data to natural mosquito populations and highlight the importance of testing field-collected mosquito populations in vector competence studies.

The results of this study contribute valuable insights into the potential risk of WNV transmission in STP. Although *Ae. aegypti* from STP demonstrated the ability to become infected and disseminate WNV, their limited capacity to transmit the virus suggests a reduced vectorial role in the event of WNV introduction. Nevertheless, continuous monitoring of both *Ae. aegypti* and other mosquito species, such as *Culex quinquefasciatus*, is recommended to better understand the potential dynamics of WNV transmission in STP. By advancing our understanding of the vector competence of *Ae. aegypti* for WNV, this research supports

global public health efforts to mitigate the spread of WNV and other emerging arboviruses in regions at risk of arboviral introductions.

Resumo

As doenças transmitidas por vetores são uma preocupação crescente de saúde pública global, especialmente em regiões tropicais e subtropicais, onde as condições climáticas favorecem a proliferação de vetores como os mosquitos. Entre as espécies de vetores mais proeminentes, *Aedes aegypti* desempenha um papel central na transmissão de arboviroses significativas, como dengue, Zika, chikungunya e febre amarela. Embora *Ae. aegypti* seja bem conhecido por transmitir esses vírus, o seu papel potencial na transmissão de outros arbovírus, como o vírus do Nilo Ocidental (WNV), é menos compreendido. O WNV é um vírus neuroinvasivo, transmitido principalmente por mosquitos do género *Culex*, que circulam o vírus entre aves, seus hospedeiros e mosquitos. No entanto, a globalização, o aumento da mobilidade humana e as aves migratórias aumentam o risco de introdução do WNV em regiões previamente não afetadas, como São Tomé e Príncipe (STP). Este estudo visa preencher uma lacuna crítica na literatura ao investigar a competência vetorial dos mosquitos *Aedes aegypti* de STP para a transmissão do WNV. Embora nenhum caso de WNV tenha sido documentado em STP até o momento, a localização estratégica da região ao longo das rotas migratórias de aves sublinha o potencial de introdução do WNV através de aves infetadas. Avaliar a competência vetorial de *Ae. aegypti* nesse contexto é essencial para avaliar riscos futuros e implementar medidas preventivas.

Neste estudo, foram conduzidas experiências laboratoriais para avaliar a capacidade dos mosquitos locais *Ae. aegypti* de adquirir, manter e transmitir o WNV. Parâmetros-chave, como taxas de infeção, disseminação e transmissão, foram avaliados em múltiplos pontos temporais (7, 14 e 21 dias pós-infeção, dpi). Os resultados revelam que *Ae. aegypti* de STP pode ser infetado com WNV, mas o potencial de transmissão permanece relativamente baixo. As taxas de infeção aumentaram progressivamente de 5% aos 7 dpi para 35% aos 21 dpi, sugerindo que, embora esses mosquitos possam adquirir o vírus, a sua suscetibilidade pode ser limitada em comparação com vetores mais eficientes, como as espécies de *Culex*. A disseminação viral, representando a propagação do vírus do intestino médio do mosquito para outros tecidos, como as glândulas salivares, atingiu o pico de 100% aos 14 dpi, mas posteriormente diminuiu para 43% aos 21 dpi. Esta diminuição sugere que, embora o vírus possa inicialmente replicar-se e espalhar-se dentro dos mosquitos, fatores como respostas imunes ou esgotamento de recursos podem limitar uma disseminação posterior ao longo do tempo. Mais criticamente, a capacidade dos mosquitos de transmitir o vírus—medida pela taxa e eficiência de transmissão—permaneceu baixa ao longo do estudo, com apenas uma pequena proporção de mosquitos a transmitir efetivamente o WNV para novos hospedeiros. Este baixo potencial de transmissão está alinhado com pesquisas anteriores que sugerem que *Ae. aegypti* é um vetor menos competente para o WNV em comparação com espécies de *Culex*, os vetores principais do vírus.

Além disso, foram realizados experimentos com a estirpe de laboratório *Aedes aegypti* Rockefeller, amplamente utilizada em pesquisas. Notavelmente, nenhum dos mosquitos desta estirpe apresentou sinais de infeção, disseminação ou transmissão de WNV. Este achado provavelmente reflete resistência inerente à infeção por WNV ou o impacto da deriva genética e consanguinidade devido ao longo tempo de criação em laboratório, o que pode reduzir a competência vetorial. Tais descobertas sublinham os desafios de extrapolar dados laboratoriais para populações naturais de mosquitos e destacam a importância de testar populações de mosquitos coletadas em campo em estudos de competência vetorial.

Os resultados deste estudo contribuem com informações valiosas sobre o risco potencial de transmissão de WNV em STP. Embora *Ae. aegypti* de STP tenha demonstrado a capacidade de ser infetado e disseminar o WNV, a sua capacidade limitada de transmitir o vírus sugere um papel vetorial reduzido no caso de uma introdução do WNV. No entanto, recomenda-se a monitorização contínua de *Ae. aegypti* e outras espécies de mosquitos, como *Culex quinquefasciatus*, para melhor entender a dinâmica potencial de transmissão do WNV em STP. Ao avançar a compreensão da competência vetorial de *Ae. aegypti* para o WNV, esta pesquisa apoia os esforços globais de saúde pública para mitigar a propagação do WNV e outros arbovírus emergentes

em regiões com risco de introduções arbovirais.

Index

Table of figures

Table of tables

List of abbreviations

ACL3	Arthropod Containment Biosafety Level 3
Ae.	*Aedes*
BSL3	Biosafety level 3
CDC	Centers for Disease Control and Prevention
CMC	carboxymethyl cellulose
Cq	cycle threshold
Cx.	*Culex*
DENV	dengue virus
DMEM	Dulbecco's Modified Eagle Medium
DNA	Deoxyribonucleic acid
DPBS	phosphate buffered saline
dpi	Days post-infection
DR	Dissemination rate
ECDC	European Centre for Disease Prevention and Control
FBS	fetal bovine serum
IHMT-Nova	Instituto de Higiene e Medicina Tropical
IR	Infection rate
MOI	Multiplicity of infections
PCR	Polymerase chain reaction
Pen/Strep	Penicillin-Streptomycin
PFU/ml	Plaque forming units per milliliter
qPCR	Quantitative PCR
RNA	Ribonucleic acid
RT-QPCR	Quantitative reverse transcription PCR
siRNA	small interfering Ribonucleic acid
STP	São Tomé and Príincipe
TR	Transmission rate
TE	Transmission efficiency
VIASEF	*In Vivo* Arthropod Security Facility
WNV	West Nile virus

1. Introduction

1.1. Overview of vector-borne diseases

Vector-borne diseases are a significant public health challenge, particularly in tropical and subtropical regions where they account for over 700,000 deaths annually and comprise more than 17% of all infectious diseases (WHO, 2020). A vector is defined as an organism that transmits pathogens—bacteria, viruses, or parasites—from one host to another. The most common vectors include mosquitoes, ticks, lice, and flies, all of which play crucial roles in the spread of various diseases(Obradovic et al., 2022).

There are two primary types of vectors: mechanical and biological. Mechanical vectors are those that transfer pathogens from an infected host, or contaminated surface, to a new host, without any biological interaction with the pathogen. These vectors merely serve as carriers, with no requirement for the pathogen to undergo development or multiplication within the vector (Foil & Gorham, 2000). For instance, a fly landing on contaminated food and then on a human can transmit pathogens mechanically from the former to the latter. In contrast, biological vectors have a more complex role in the transmission of disease-causing infectious agents. In these vectors, the pathogen not only survives but often undergoes significant changes, that might result in its multiplication (increasing its numbers) or a transformation to another stage in its life cycle. This biological interaction is essential for the pathogen's development and eventual transmission to a new host(Verwoerd, 2015).

Among the myriad of vector-borne diseases, those transmitted by mosquitoes are particularly notorious, with *Aedes aegypti* being a primary vector of several viral agents that cause diseases including dengue, Zika, chikungunya, and yellow fever. Effective control of *Ae. aegypti* populations is crucial in mitigating these diseases. Various strategies have been developed for this purpose, including population control through environmental management, genetic modifications to render the mosquito incapable of pathogen transmission, the use of insecticides, the use of sterile males with a *Wolbachia* strain and the application of non-chemical larvicides (Weeratunga et al., 2017;WHO, 2020; Kain et al., 2022; Hugo et al., 2022).

Understanding vector competence, which is the ability of a vector to acquire, maintain, and transmit a pathogen, is fundamental to assessing the risk of disease spread. This competence involves a series of interactions between the vector and the pathogen, including the mosquito's susceptibility to infection, the pathogen's ability to replicate within the vector, and the efficiency of transmission to a new host (Kain et al., 2022).Research into the vector competence of *Ae. aegypti* is particularly important in regions like São Tomé and Príncipe (STP), where unique environmental and ecological factors may influence transmission dynamics. Given the island's tropical climate, a suitable habitat for *Ae. aegypti*, understanding how this mosquito species interacts with pathogens can inform about the need for supplementary control strategies. This knowledge is critical not only for local public health efforts but also for global strategies aimed at preventing the spread of

vector-borne diseases.

1.2. Importance of *Aedes aegypti*

Aedes aegypti, along with *Aedes albopictus*, is among the most critical vectors of arthropod-borne viruses (arboviruses), due to their proven competence in transmitting four major arboviruses: dengue, Zika, chikungunya, and yellow fever (Lwande et al., 2020). The global impact of these diseases is profound, both in terms of public health and economic burden, making *Ae. aegypti* a key focus of vector control efforts worldwide. In south America in 2024 there were approximately 11 million cases of Dengue fever (PAHO, 2024). For instance, the chikungunya outbreak in Brazil in 2016 had severe repercussions, costing the country approximately 2.3 billion reais in healthcare expenses, lost productivity, and other related costs (Teich et al., 2017). Similarly, the annual cost of dengue in Puerto Rico from 2002 to 2010 was estimated at 46.45 million dollars, underscoring the persistent economic strain caused by this disease (Halasa et al., 2012). The Zika virus, which gained international attention during the 2015-2016 outbreak, also inflicted significant health and economic damage. In Brazil alone, there were approximately 1.7 million reported cases of Zika virus infection between January 2015 and November 2016. The outbreak was particularly alarming due to the associated increase in congenital anomalies, such as microcephaly, leading to long-term healthcare needs and social support for affected families (de Oliveira et al., 2017).

These examples emphasize the burden that *Ae. aegypti* can impose on societies, particularly in tropical and subtropical regions where these mosquitoes thrive. The species' ability to adapt to urban environments, combined with its preference for human blood and its effectiveness in transmitting multiple viruses, makes *Ae. aegypti* an especially formidable public health threat.

Given its critical role in the transmission of several major arboviruses, understanding the vector competence of *Ae. aegypti* is crucial for developing and implementing effective control measures. By studying how this mosquito species interacts with different pathogens, including its ability to acquire, maintain, and transmit viruses, researchers can better predict and mitigate the spread of these devastating diseases.

1.3. *Aedes aegypti*

1.3.1. Biology and distribution of *Aedes aegypti*

Aedes aegypti (Linnaeus, 1762) (Figure 1) is a mosquito species whose name aptly reflects its historical association with human habitation and the discomfort it causes. The genus name *Aedes* is derived from the Greek word meaning "unpleasant" or "odious" (Gil-Santana et al., 2021) highlighting the nuisance

this mosquito represents. The species name *aegypti* refers to its initial identification in Egypt, underscoring its early recognition as a significant pest.

Figure 1. Graphic illustration of *Ae. aegypti* species. Retrieved from: https://www.wrbu.si.edu/vectorspecies/mosquitoes/*aegypti*.

The life cycle of *Ae. aegypti* comprises four distinct stages: egg, larvae (with four instar phases), pupae, and adult mosquito (Figure 2).

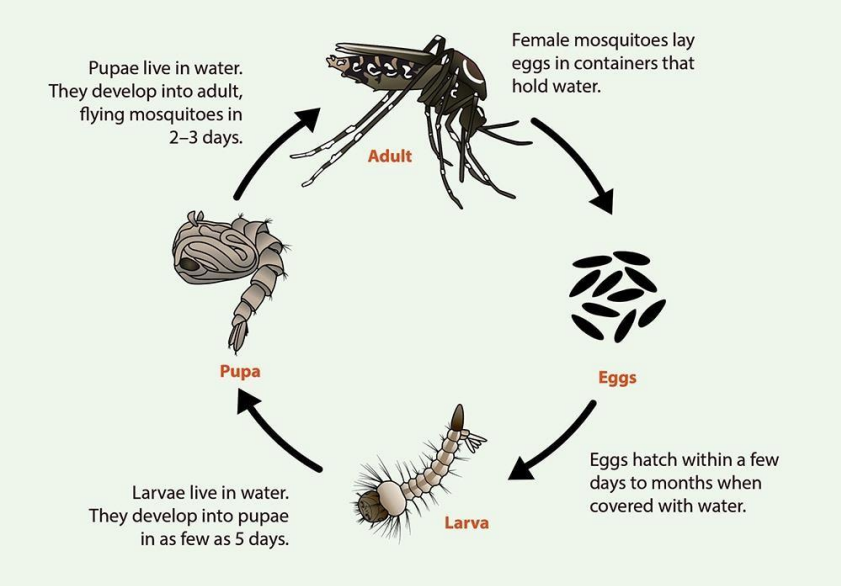

Figure 2. Life cycle of *Ae. aegypti* mosquito. Retrieved from: https://www.cdc.gov/mosquitoes/about/life-cycle-of-aedes-mosquitoes.html.

The adult mosquito is particularly notable for its distinctive lyre-shaped pattern of white scales on its mesonotum, a key identifying feature (Supriyono et al., 2023). This species is further classified into two subspecies: *Aedes aegypti formosus* and *Aedes aegypti aegypti*.

- *Aedes aegypti formosus* primarily inhabits sylvatic, or forested, environments in Africa. This subspecies is less associated with human populations and typically does not play a significant role in disease transmission to humans.
- In contrast, *Aedes aegypti aegypti* has evolved to thrive in urban settings, exhibiting a strong preference for human odors. This adaptation facilitates its role as a primary vector for several arboviruses (Powell & Tabachnick, 2013; McBride et al., 2014).

Ae. aegypti was transported to the Americas from Africa around the 1500s by the slave trade, the first reliable account of Yellow Fever in the New World was in Havana in 1648, it returned to the Mediterranean ports around 1800s where it remained until 1950, where it was eradicated with the use of DDT. It spread to Asia and Australia after the opening of the Suez Canal (Powell et al., 2018).

Geographically, *Ae. aegypti* is predominantly found in tropical and subtropical regions, where warm and humid climates provide ideal conditions for its breeding and survival. However, this species has also been documented in certain temperate regions, demonstrating its ability to expand its range under favorable conditions. Notable examples include its establishment in Madeira Island, Portugal (Almeida et al., 2007), and Sochi, Russia (Iunicheva et al., 2008) and Cyprus (Vasquez et al., 2023). These cases exemplify the mosquito's capacity to adapt to different environments, raising concerns about its potential to invade new territories as global temperatures rise.

Beyond its well-established role in transmitting dengue, Zika, chikungunya, and yellow fever viruses, *Ae. aegypti* is also a competent vector for other significant viruses, further underscoring its public health importance. For example, the Mayaro virus (Wiggins et al., 2018). The transmission of WNV by *Ae. aegypti* is of particular interest, as studies have shown that, while the transmission rate is relatively low, it is not negligible. For instance, research has demonstrated that the transmission rate for the WNV strain Crow 379-99 with the Rockefeller strain of *Ae. aegypti* was less than 16% (Turell et al., 2001). Although these rates may seem modest, they are sufficient to warrant concern, especially in areas where this mosquito species is prevalent and where other more efficient WNV vectors might not be present.

The knowledge regarding the biology and distribution of *Ae. aegypti* is crucial for developing effective control strategies, particularly as this species continues to expand its geographical range. Its ability to colonize new areas poses an increasing threat to global public health, necessitating ongoing research and vigilant surveillance to prevent and mitigate the spread of the diseases it transmits.

1.4. West Nile Virus

1.4.1. Overview of West Nile Virus

West Nile Virus is an enveloped, positive-sense, single-stranded RNA virus, approximately 50

nanometers in diameter (Londono-Renteria & Colpitts, 2016). It belongs to the family *Flaviviridae*, a group known for including other significant pathogens such as dengue, Zika, and yellow fever viruses (Karim & Bai, 2023). The virus was first isolated in 1937 from the blood of a febrile patient in the West Nile District of Uganda, which gave the virus its name (Smithburn et al., 1940). Since its discovery, WNV has spread globally, becoming one of the most important causative agents of viral encephalitis in humans, it is part of the Japanese encephalitis serocomplex (Khare & Kuhn, 2022). The virus is endemic in many parts of Africa, Europe, the Middle East, and the Americas (Lim et al., 2011) and Australia (Department of health, 2024). Although most human infections (approximately 75%) are asymptomatic, about 25% of infected individuals develop a mild illness known as West Nile fever, characterized by symptoms such as fever, headache, body aches, and occasionally a skin rash or swollen lymph glands (Petersen et al., 2013). A small percentage (less than 1%) of infected individuals develop neuroinvasive disease, which can lead to severe conditions such as encephalitis, meningitis, or acute flaccid paralysis. This severe form is more common in older adults, and among those who develop neuroinvasive disease the case fatality rate can be as high as 10% (Petersen et al., 2013).

West Nile virus is maintained in nature through a zoonotic cycle that primarily involves mosquitoes and birds (Figure 3). WNV can be transmitted by mosquito bite to humans, horses and crocodiles, apart from that it can also be transmitted by blood transfusion or organ transplant. Birds act as amplifying hosts, meaning they can develop high levels of viremia sufficient to infect mosquitoes during feeding. However, the efficiency of transmission can vary by bird species. For example, studies have shown that despite similar viremia levels, mosquitoes tend to get more infected in robins than grackles (Vaughan et al., 2022). The risk areas for WNV are connected globally through migratory bird pathways. (García-Carrasco et al., and collaborators (2023) identified 61 bird species that could potentially contribute to the intercontinental spread of WNV. Notably, there has been evidence of possible WNV strain exchanges between Senegal and Europe due to migratory birds (Ndione et al., 2022). In STP, there are 186 documented species, including at least four non-breeding migratory species of birds (De Lima & Melo, 2021) in which at least one breeds in northern Europe (BirdLife International,2024), which could facilitate the introduction and dissemination of WNV into the region through their migratory patterns.

Humans and other mammals, however, are considered "dead-end" hosts because the viremia levels they develop are typically too low to contribute to the transmission cycle (Colpitts et al., 2012). Among mosquitoes, the genus *Culex* is the most common vector for WNV, particularly species such as *Culex pipiens* and *Culex quinquefasciatus*, which are highly efficient at transmitting the virus in various regions (Colpitts, 2016). *Culex quinquefasciatus* is the vector of multiple pathogens like the St. Louis encephalitis virus, where it is the principal vector in the southern U.S, Rift Valley fever virus and *Wuchereria Bancrofti* (UF, 2025).

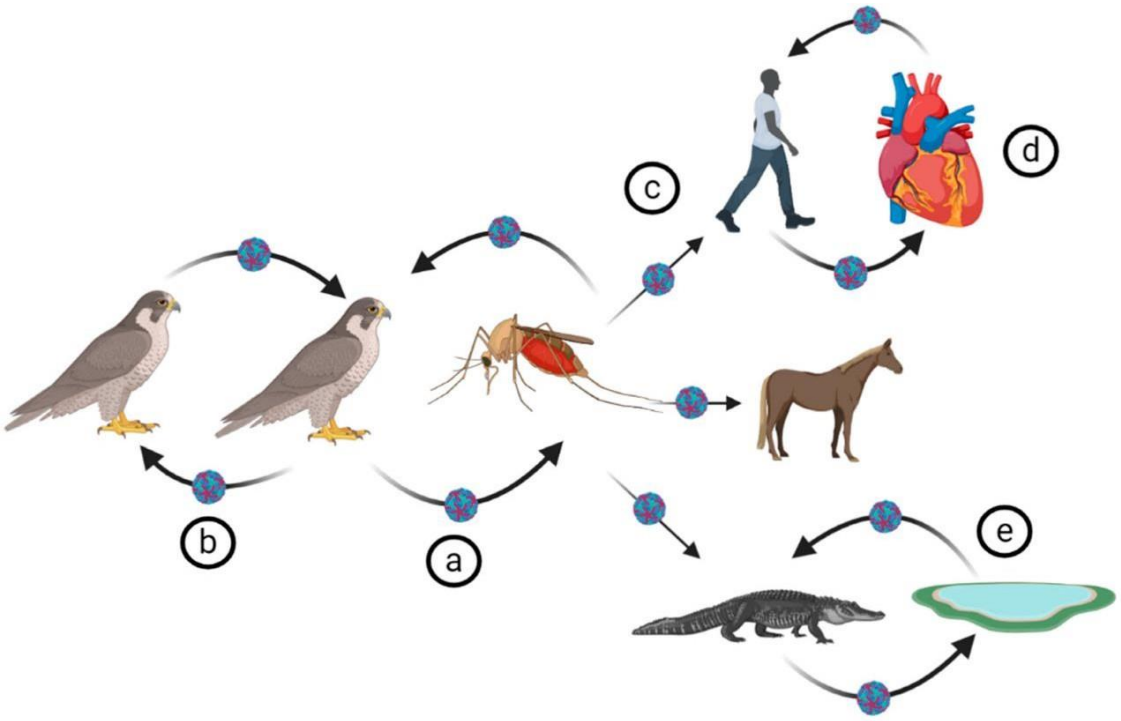

Figure 3. WNV life cycle and transmission. (A) WNV maintenance between birds (reservoir) and competent mosquito vector, mainly *Culex* sp.; (B) WNV transmission directly between birds in commercial farm settings; (C) WNV transmission to various hosts (human, horse, crocodile) via mosquito bite; (D) WNV transmission via blood transfusion and organ transplant in human; (E) WNV infection in crocodile through WNV contaminated water. Source: (Habarugira et al., 2020).

The WNV genome (Figure 4) is approximately 11,000 nucleotides long and consists of a 5'-capped RNA which tends to form a stem-loop structure at the 3' end (Brinton, 2013). The genome encodes three structural proteins (C, prM/M, and E) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5), which are synthesized from a single polyprotein precursor that is cleaved into individual components during viral replication (Lindenbach & Rice, 2003a). The structural proteins are essential for virus assembly and entry into host cells, while the nonstructural proteins are involved in RNA replication, immune evasion, and modulation of the host cell environment (Lindenbach & Rice, 2003b).

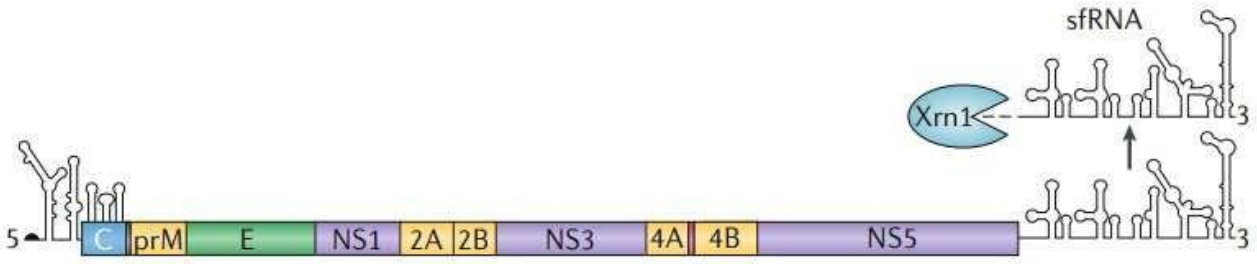

Figure 4. The genome and encoded proteins of ZiKV. Adapted from: (Liu et al., 2019).

Upon entering a host, WNV initiates infection by binding to cell surface receptors and entering cells via receptor-mediated endocytosis (Mukhopadhyay et al., 2005a). The virus utilizes lipid rafts—cholesterol-rich microdomains in the plasma membrane—to facilitate its entry into the cell (Medigeshi et al., 2008). Once inside the cell, the endosomal membrane fuses with the viral envelope, triggered by the acidic environment, releasing the viral RNA genome into the cytoplasm. This RNA is then translated into a polyprotein, which is subsequently cleaved by viral and host proteases to generate the individual structural and nonstructural proteins necessary for viral replication(Mukhopadhyay et al., 2005b).

The replication of WNV involves the synthesis of a complementary negative-sense RNA, which serves as a template to produce new positive-sense genomes. These genomes are assembled with structural proteins within the endoplasmic reticulum to form immature virions. The virions undergo further maturation processes as they transit through the host cell's secretory pathway, eventually being released from the cell via exocytosis (Lim et al., 2011). The efficient replication and release of WNV in both vertebrate and invertebrate hosts underpins its successful transmission and persistence in diverse ecological niches.

1.4.2. Epidemiology of West Nile virus and outbreaks

West Nile Virus is recognized as the most widespread arbovirus globally, significantly contributing to the burden of viral encephalitis across diverse regions (Ciota, 2017). Phylogenetically, WNV is classified into nine different lineages (Lu et al., 2024) with lineage 1 and 2 being the most pathogenic and widespread. Lineage 2 is, in the European continent, the principal contributor to the occurrence of serious cases although not being the most prevalent (Mencattelli et al., 2023). The extensive distribution of WNV has led to numerous outbreaks worldwide, each varying in scale and impact. Notable outbreaks include the 1996 epidemic in Romania, which marked one of the earliest significant events in Europe, and the subsequent outbreaks in southern Russia in 1999, which highlighted the virus's spread across Eastern Europe (HAYES, 2001). The introduction of WNV into the United States in 1999, first identified in New York City, represented a major turning point in the global epidemiology of the virus.

This event led to rapid dissemination across North America, resulting in thousands of cases and establishing WNV as an endemic pathogen in the region (HAYES, 2001). According to (CDC, 2024) since its first recorded cases in 1999 until 2023 in the United States of America there has been over 59.000 cases.

In addition to these well-documented outbreaks, WNV has shown a capacity for unpredictable epidemiological patterns. For example, in Sudan, an unusual outbreak occurred between May and August 2002, primarily affecting children aged five years or younger (Depoortere et al., 2004). This deviation from the typical epidemiological pattern—where severe cases are more common in older adults—underscores the variability in WNV's clinical presentation and impact across different populations.

In Europe, WNV has also caused significant public health concerns. A major outbreak occurred in Greece in 2010, where 17% of patients diagnosed with West Nile neuroinvasive disease (WNND) succumbed to the illness. This outbreak highlighted the severe impact WNV can have on affected populations, particularly in regions where the virus is newly introduced or re-emerging (Danis et al., 2011). Similarly, an outbreak in Italy in 2018, although smaller in scale with only nine reported cases, saw a disproportionately high incidence of neuroinvasive disease, with eight of the nine cases developing WNND. This indicates that even small outbreaks can have severe outcomes, particularly among vulnerable populations, with comorbidities like Chronic Lymphocitic Leucemia and Non-Hodgkin Lymphoma (Lupia et al., 2022).

The persistence of WNV in Europe and North America continues to be a public health concern. As of December of 2024, there were 19 countries in Europe that reported human cases of WNV, reflecting the virus's ongoing transmission in the region (ECDC, 2024). In the United States, by August 27, 2024, 289 human cases had been reported, illustrating the continued threat WNV poses in areas where it has become endemic (CDC, 2024).

São Tomé and Príncipe, the focus of this thesis, has not yet experienced a documented outbreak of WNV (Figure 5). However, given the island nation's geographical location and the presence of competent mosquito vectors (*Cx. quinquefasciatus*, *Ae. aegypti*, *Ae. albopictus(Bohers et al., 2024)*), the potential for WNV introduction and subsequent transmission remains a concern. Monitoring and preparedness are crucial to preventing an outbreak and mitigating its impact should the virus be introduced.

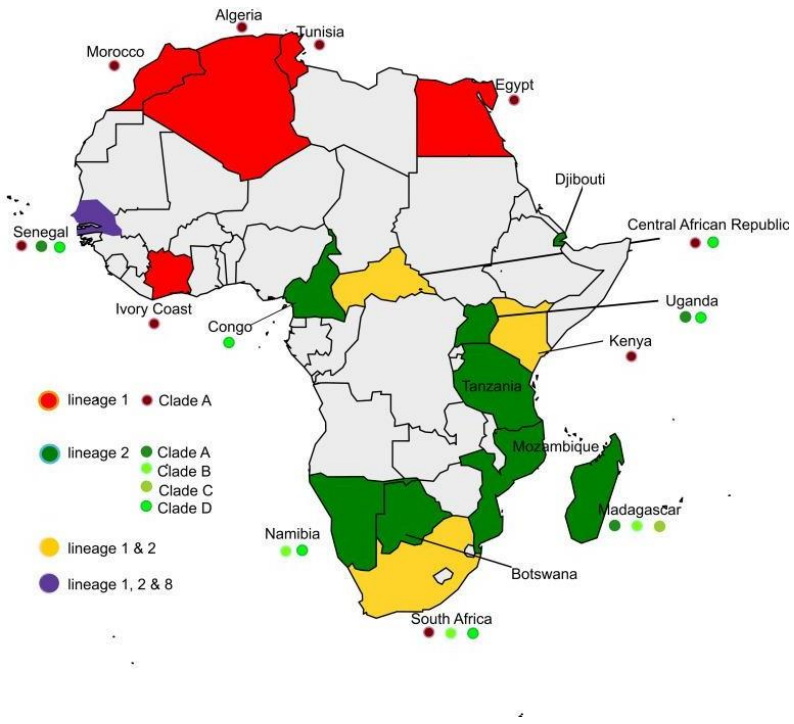

Figure 5. West Nile virus in 17 countries in the African continent. Source: (Mencattelli et al., 2022).

In neighboring countries within the Gulf of Guinea, including Congo and Cameroon, WNV has been

detected, with various clades of lineage 2 circulating in these regions (Mencattelli et al., 2022). Given STP's

geographical proximity to these areas, the risk of WNV introduction is a growing concern, especially with migratory birds and the increasing globalization as a potential way for the introduction of the virus. To effectively assess this risk, it is essential to evaluate the vector competence of local mosquito populations, as this plays a crucial role in determining vectorial capacity for WNV transmission.

1.5. Vectorial capacity and vector competence

1.5.1. Vectorial capacity

Vectorial capacity (Figure 6) is a comprehensive measure that defines the potential of a mosquito population to transmit a particular pathogen within a specific environment. It encompasses several factors, including the abundance of the mosquito population, the rate of mosquito feeding on hosts, the survival rate of mosquitoes, and the duration of extrinsic period of virus development in the mosquito (Smith et al., 2012). This measure provides a broad understanding of the transmission dynamics of vector-borne diseases.

Vector competence is a key trait within vectorial capacity (Beerntsen et al., 2000), focusing specifically on the intrinsic ability of a mosquito to acquire, maintain, and transmit a pathogen to a vertebrate host (V. Y. Wu et al., 2022). While vectorial capacity considers external factors such as environmental conditions and mosquito behavior, vector competence is determined by the biological interactions between the mosquito and the pathogen.

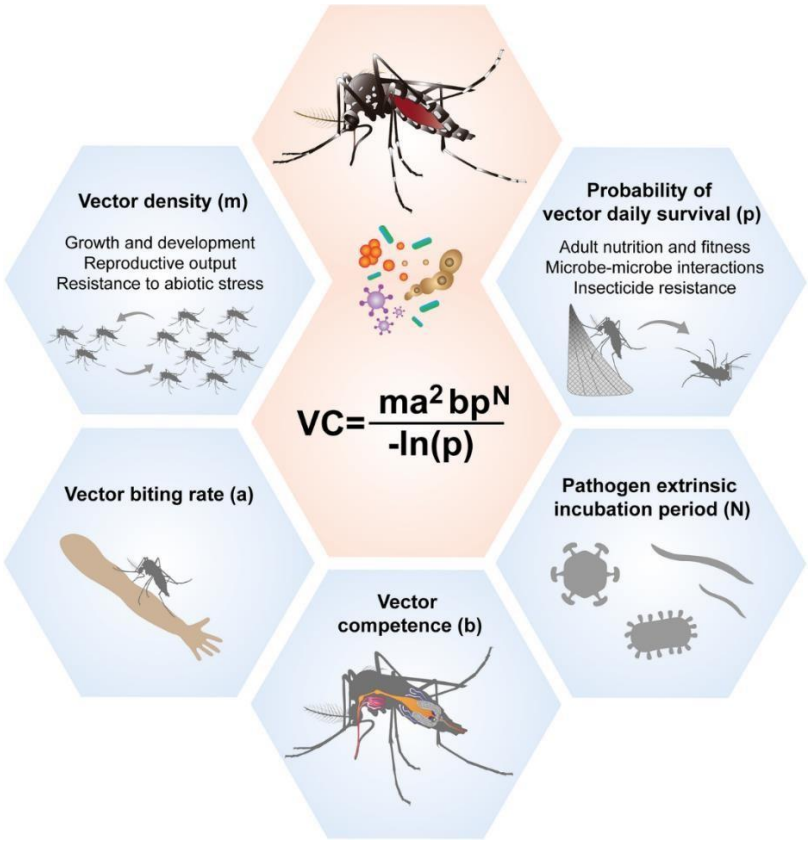

Figure 6. Graphic illustration of the concept of vectorial capacity in the context of mosquito-borne disease transmission. The equation integrates key components influencing vectorial capacity – (a) vector biting rate, frequency of host biting by mosquitoes; (b) vector competence, mosquito's ability to acquire, maintain and transmit a pathogen; (N) pathogen extrinsic incubation period, time required for the pathogen to become transmissible within the vector; (p) probability of vector daily survival, affected by factors like nutrition, fitness and insecticide resistance; (m) vector density, determined by growth, development, reproductive output and resistance to abiotic stress. Source: (Cansado-Utrilla et al., (2021).

1.5.2. Vector competence: mechanisms and factors

Vector competence is defined as the intrinsic ability of a mosquito to acquire, maintain, and transmit a virus to a vertebrate host (V. Y. Wu et al., 2022). This process involves several critical stages: the virus must first successfully enter the mosquito, then replicate within it, disseminate to various tissues, and finally be released at high enough concentrations to infect a new host during subsequent blood feeding (V. Y. Wu et al., 2022). The efficiency of each of these processes determines a mosquito's potential to act as a vector for specific pathogens.

Several factors influence vector competence, making it a complex trait governed by both environmental and biological determinants. Temperature is one of the most significant environmental factors; it affects the rate of viral replication within the mosquito and can alter the extrinsic incubation period (EIP), which is the time required for the virus to reach the salivary glands and become transmissible (Winokur et al., 2020). Higher temperatures generally shorten the EIP, leading to quicker transmission cycles, while lower temperatures prolong the EIP and reduce transmission efficiency. Humidity levels also influence mosquito survival and feeding behavior, thereby affecting their capacity to transmit pathogens. Seasonal changes can alter mosquito population dynamics and vector-pathogen interactions, necessitating continuous monitoring of environmental conditions to predict outbreak risks accurately.

A critical aspect of vector competence involves several physiological barriers within the mosquito that can impede the progression of the virus from ingestion to transmission (Figure 7):

- **Midgut Infection Barrier**: After a mosquito ingests an infected blood meal, the virus must first infect the midgut cells. Some mosquitoes possess a midgut infection barrier (peritrophic membrane) that depends on siRNAs, preventing the virus from infecting these cells and halting further transmission. If the virus overcomes this barrier, it can replicate within the midgut cells (Agarwal et al., 2017).
- **Midgut Escape Barrier**: Even if the virus successfully infects the midgut, it must escape into the hemocoel (the mosquito's body cavity) to spread to other organs. The midgut escape barrier can prevent the virus from leaving the midgut, thus stopping its dissemination and preventing further infection of secondary tissues like the salivary glands (Merwaiss et al., 2021).

- **Salivary Gland Infection Barrier**: If the virus reaches the hemocoel, it must infect the salivary glands to be transmitted to a new host during a mosquito's next blood meal. The salivary gland infection barrier can prevent this, and only viruses that overcome this barrier will be transmitted. The effectiveness of this barrier can decrease over time, but it remains a significant bottleneck in viral transmission (Jennings & Kay, 1999).

Additionally, the dose of the virus ingested can influence infection and transmission rates, older colonies may exhibit decreased vector competence due to factors such as inbreeding and reduced genetic diversity that affects fitness (Ross, Endersby-Harshman, et al., 2019). Moreover, the larval environment, including nutrition and exposure to insecticides, can significantly impact the adult mosquito's susceptibility to arboviral infections. For example, inadequate nutrition during the larval stage can result in weaker adults with altered immune responses, potentially reducing their vector competence (Alto & Lounibos, 2013). Similarly, sublethal exposure to insecticides during development can stress the mosquitoes, affecting their ability to harbor and transmit viruses (Alto & Lounibos, 2013).

Genetic factors also play a pivotal role in determining vector competence. Studies have shown that mosquito populations with higher baseline immune activity are less susceptible to viral infections. For instance, gene expression analysis has revealed that refractory mosquitoes—those resistant to viral infections—exhibit elevated levels of immune-related gene expression compared to susceptible populations (Bonizzoni et al., 2012). This genetic variation underscores the importance of understanding the molecular interactions between the virus and the vector to develop targeted control strategies.

The microbiota within the mosquito gut also affects vector competence (Figure 7). Commensal bacteria and other microorganisms can modulate the mosquito's immune responses and influence viral replication (Rainey et al., 2014). For example, certain bacterial species have been shown to enhance or inhibit the replication of arboviruses within mosquitoes (Ciota et al., 2013); (P. Wu et al., 2019). Understanding the interactions between the mosquito microbiota and viral pathogens can provide novel insights into vector competence and potential strategies for biological control.

The strain and genotype of the virus also play a crucial role in vector competence. Different WNV strains exhibit varying levels of infectivity and replication efficiency in mosquito vectors (Fall et al., 2014). Viral genetic mutations can influence the ability of the virus to overcome mosquito immune defenses and exploit cellular machinery for replication (Van Slyke et al., 2015). Co-evolutionary dynamics between specific mosquito populations and virus strains can result in unique interactions that affect transmission potential (Ciota et al., 2013). Studies have shown that certain WNV genotypes are more efficiently transmitted by specific mosquito species, highlighting the importance of understanding viral genetic factors in vector competence assessments(Fall et al., 2014; Moudy et al., 2017).

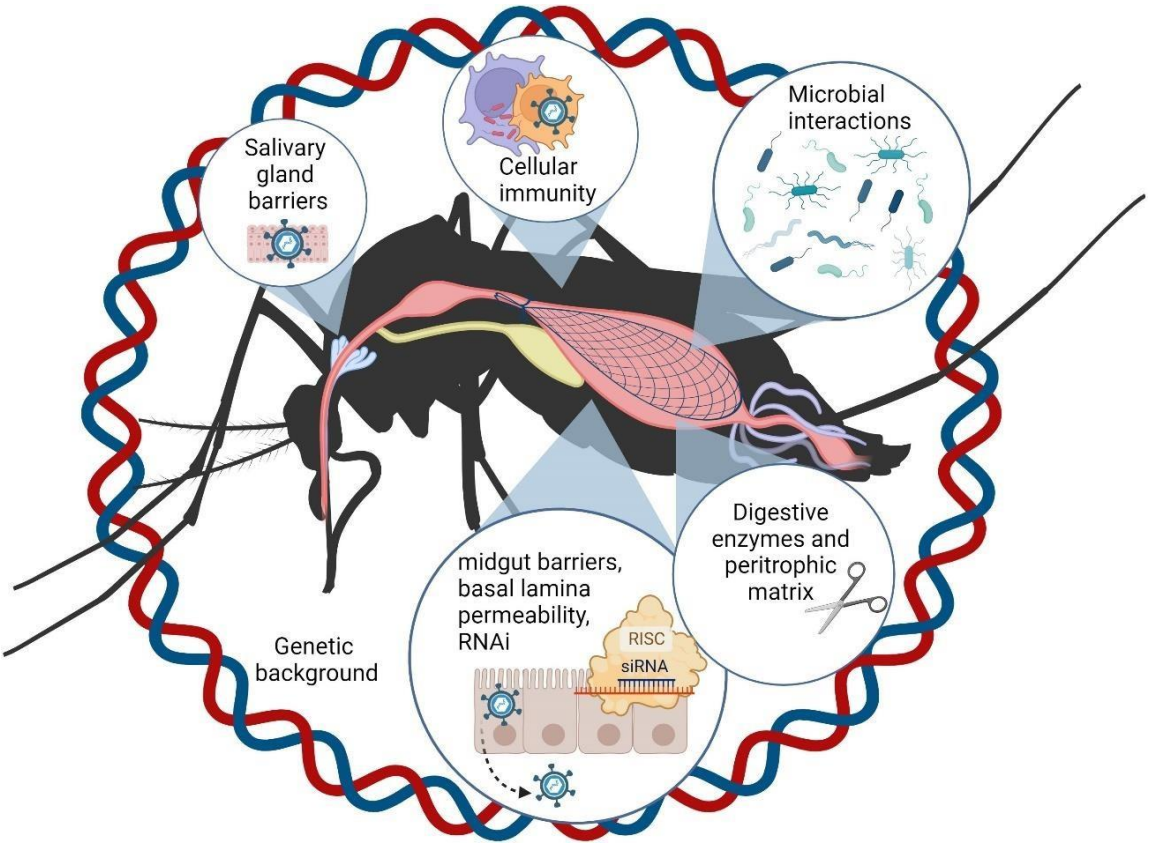

Figure 7. Intrinsic mosquito factors that influence vector competence and viral evolution. Source: (Lewis et al., (2023).

Vector competence is a multifaceted trait influenced by a combination of environmental factors, mosquito biology, viral strain variation and genetic background. The efficiency with which a pathogen can infect and traverse these barriers determines its ability to establish infection within the vector and to be transmitted to a new host. Understanding these mechanisms is crucial for predicting and managing the transmission dynamics of arboviruses, particularly in regions where vectors like *Ae. aegypti* play a significant role in disease spread.

1.6. Study region: São Tomé

São Tomé and Príncipe are an island nation located in the Gulf of Guinea, off the western coast of Central Africa, near the equator. The country's climate is characterized by tropical conditions, with annual average temperatures ranging between 22°C and 26°C and an average annual precipitation of approximately 900 mm (Climate Change Knowledge Portal, 2023). São Tomé and Príncipe experience two distinct seasons: the wet season, which occurs from October to May, and the dry season, which spans from June to September. The tropical climate of STP, with its relatively consistent warm temperatures, provides an ideal environment for the proliferation of mosquitoes and enhances the transmission efficiency of arboviruses, as lower temperatures are known to negatively impact transmission rates (Heitmann et al., 2018).

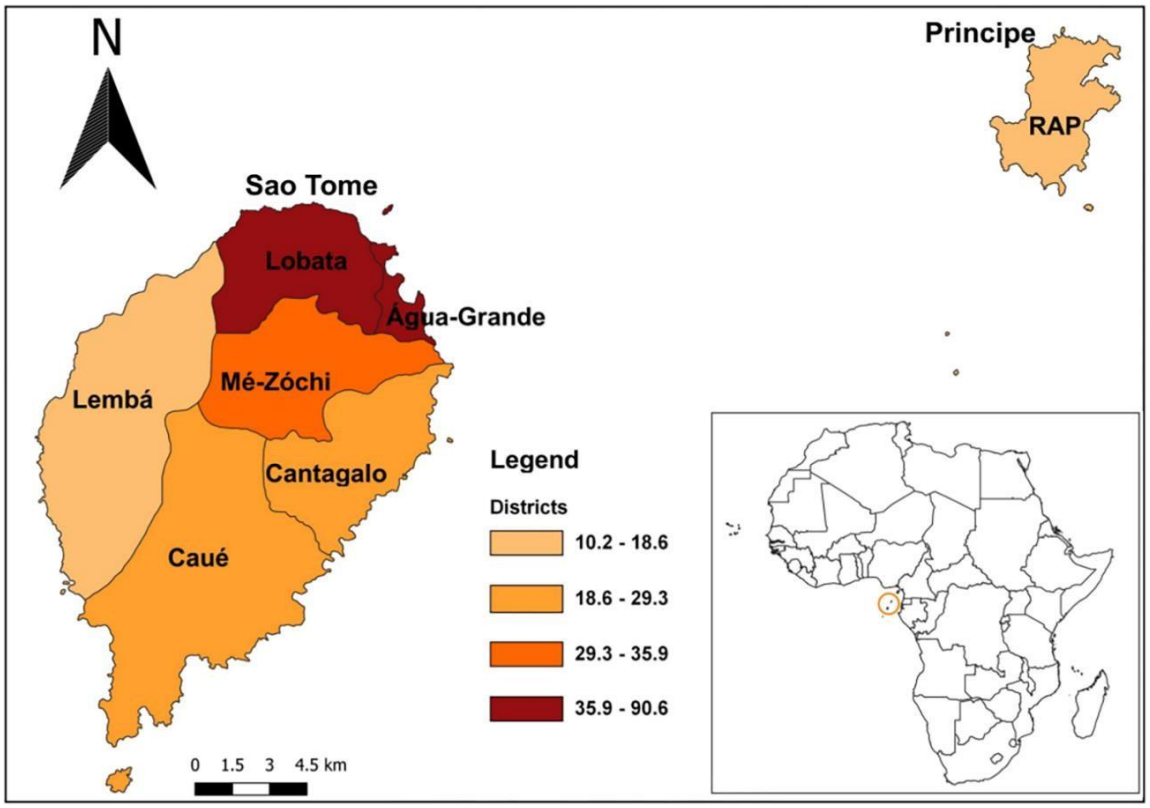

Figure 8. Map of STP indicating the seven health districts. Colors indicate population density by health district. Source: (Kamgang et al., (2024).

São Tomé and Príncipe were selected as the study location for this research due to the presence of *Ae. aegypti*, a well-established resident mosquito species on the islands (Kamgang et al., 2024). *Aedes aegypti* is a primary vector of several significant arboviruses, making it a critical focus for studies on vector competence and disease transmission. While malaria in STP has shown a declining trend in both incidence and mortality, it remains a persistent public health challenge that will be difficult to fully eradicate (Wang et al., 2022). This ongoing burden underscores the importance of continued vector control efforts, particularly as the emergence and re-emergence of other vector-borne diseases, such as dengue, pose additional threats to public health. In 2022, STP experienced its first reported outbreak of dengue fever, highlighting the growing significance of arboviral diseases in the region. During this outbreak, entomological surveys revealed the widespread presence of both *Ae. aegypti* and *Ae. albopictus* across all seven districts of the islands, with *Ae. aegypti* being particularly prevalent in the most populous district (Kamgang et al., 2024). Furthermore, seroprevalence studies conducted between 2003 and 2004 indicated the presence of DENV (dengue virus) antibodies among pregnant women in STP, suggesting that dengue virus transmission has been ongoing in the country, even before the documented outbreak(Yen et al., 2016).

This research aims to provide valuable insights into the transmission dynamics of WNV within this unique island environment. Although *Ae. aegypti* is not the main vector of WNV, it may serve as a bridge vector in STP, transmitting the virus between birds and humans. Understanding the potential role of *Ae. aegypti* in

facilitating WNV spillover (Linthout et al., 2024) to human populations is crucial for informing public health strategies and enhancing preparedness against the possible emergence of WNV in the region.

1.7. Objectives of this study

Currently, there is a gap in the literature regarding the vector competence of *Ae. aegypti* for WNV in STP. This study aims to address this gap by assessing the vector competence of *Ae. aegypti* for WNV within this unique geographical context. This involves determining the ability of local *Ae. aegypti* mosquitoes to acquire, maintain, and transmit WNV, thus providing essential data on the risk of WNV transmission in STP. This study involved laboratory-based experiments to measure infection, dissemination and transmission rates within the mosquito population. By gathering this data, this study will offer insights into the potential risk of WNV transmission in STP.

The findings from this study will provide valuable insights into the epidemiological risks associated with *Ae. aegypti* in STP and will support the formulation of evidence-based strategies to prevent and control potential WNV outbreaks in the region.

2. Materials and Methods

Vero E6 cells were cultured and used for the production and quantification of West Nile Virus (WNV) via plaque assays, while *Ae. aegypti* mosquitoes were reared, infected, and analyzed through RT-qPCR for WNV infection, dissemination, and transmission rates, with statistical analysis performed to assess significant differences.

2.1. Cell culture

2.1.1. Cell types and medium used

For the production and quantification of WNV via plaque assays, African Green monkey kidney cells (Vero E6) were employed due to their high susceptibility to viral infections and robust performance in virological assays. These cells were cultured in vented T25 and T75 cell culture flasks using Dulbecco's Modified Eagle Medium (DMEM, Gibco), which was supplemented with 10% Fetal Bovine Serum (FBS, Sigma-Aldrich) and 1% Penicillin-Streptomycin (Pen/Strep, Sigma-Aldrich) to ensure optimal growth and viability.

2.1.2. Cells conditions and maintenance

The Vero E6 cells were maintained under standard cell culture conditions in a humidified incubator set to 37°C with 5% CO_2. When the cells reached near-confluency, subculturing was necessary to prevent overgrowth and maintain cell health.

For subculturing, the growth medium was first completely removed from the flask. The cells were then washed with 5 ml of Dulbecco's Phosphate Buffered Saline (DPBS,Gibco) for T25 flasks and 10 ml for T75 flasks to remove any residual FBS that could inhibit trypsin activity. After approximately 30 seconds, the DPBS was discarded, and 3 ml of 1x Trypsin-EDTA solution (Gibco) was added to the T25 flasks, while 5 ml was added to the T75 flasks. The flasks were then incubated at 37°C with 5% CO_2 for 3 minutes to facilitate cell detachment.

Once the cells were detached, 3 ml of DMEM containing 5% FBS was added to the T25 flasks and 5 ml to the T75 flasks to neutralize the trypsin. The cell suspension was then diluted at ratios of 1:10 or 1:5, depending on the desired seeding density, and transferred to new flasks containing fresh, pre-warmed medium.

2.2. Virus Production and quantification

2.2.1. Virus Strain and production

The WNV strain used in this study was the PT6.39 strain (GenBank accession number AJ965630.2) which belongs to the lineage 1 more specifically in the cluster Morocco (Esteves et al., 2005). The virus was propagated from a viral stock with a concentration of 3.6×10^7 plaque-forming units (PFU)/ml.

To produce the virus, confluent Vero E6 cells with a cell viability of 90 % were infected in two T75 flasks with a multiplicity of infection (MOI) of 0.1 and 0.01, respectively. The virus was allowed to adsorb to the cells by incubating the flasks at 37°C with 5% CO_2 for three days.

After the incubation period, the entire content of each flask, including both the supernatant and detached cells, was collected into 15 ml conical tubes. The tubes were then centrifuged at 3000g for 5 minutes to pellet cellular debris. The resulting supernatant, containing the virus, was carefully transferred into cryogenic storage vials (Thermo Fisher Scientific) and stored at -80°C for future use.

2.2.2. Virus quantification

Viral titration was performed using a plaque assay with Vero E6 cells, following previously published protocols(Brien et al., 2013)(; McAuley & Beasley, 2016) with slight modifications.

After subculturing from T25 flasks, the Vero cells were diluted at a ratio of 1:20 and seeded into 12-well culture plates. The plates were incubated overnight at 37°C with 5% CO_2 to allow the cells to form a monolayer that was approximately 90% confluent by the following day.

The next day, serial tenfold dilutions of the stored virus were prepared, ranging from 10^{-1} to 10^{-6}. These dilutions were made in serum-free 1x DMEM by mixing 200 µl of the virus stock with 1800 µl of DMEM in 2 ml centrifuge tubes (Eppendorf). Following this, 400 µl of each dilution was added to the respective wells of the 12-well plates, ensuring that the virus was evenly distributed across the cell monolayers. The plates were then incubated for one hour at 37°C with 5% CO_2, with gentle rocking every 20 minutes to ensure uniform viral adsorption.

After the incubation period, without removing the inoculum, 1 ml of overlay medium was added to each well. The overlay consisted of a 1:1 mixture of 2X DMEM containing 2% FBS and 2% Pen/Strep with 1.5% high-viscosity carboxymethyl cellulose (CMC, Sigma-Aldrich). The plates were left undisturbed for 5-10 minutes to allow the overlay to settle, then incubated at 37°C with 5% CO_2 for three days to allow plaque formation.

On the third day, the cells were fixed by adding 1 ml of 3.7% formaldehyde to each well, allowing the fixative to act for approximately one hour. Following fixation, the formaldehyde and overlay were carefully discarded, and the wells were washed with phosphate-buffered saline (PBS, Gibco) to remove any remaining overlay material. After a brief wash with PBS, the wells were rinsed with a gentle stream of running water.

The cells were then stained with crystal violet for 15 minutes to visualize the plaques. After staining, the wells were again washed with water to remove excess stain. The resulting plaques were observed and counted manually under a light microscope. The viral titer, expressed as plaque-forming units per milliliter (PFU/ml), was calculated using the following formula:

$$PFU/mL = \frac{Number\ of\ plaques \times Dilution\ Factor}{Volume\ of\ Inoculum\ (mL)}$$

This formula allowed for the accurate determination of viral concentration in the original stock.

2.3. Mosquito Rearing

2.3.1. Mosquito collection, identification and maintenance

Aedes aegypti strain Rockefeller (MRA-734) was obtained from BEI Resources (https://www.beiresources.org/Catalog/BEIVectors/MRA-734.aspx) in October 2022 and installed at "*In Vivo* Arthropod Security Facility" belonging to Instituto de Higiene e Medicina Tropical of Universidade Nova de Lisboa (VIASEF, IHMT-NOVA), a high security facility to rear and manipulate autochthonous and invasive species available at IHMT. We aim to characterize the WNV susceptibility of this Rockefeller colony, using a viral dose previously effective in *Cx. quinquefasciatus* colony from Cape Verde available at VIASEF.

Eggs of mosquitoes from STP were collected using ovitraps strategically placed in various locations across São Tomé city during the summer of 2023. The collected eggs were transported to VIASEF and allowed to hatch into larvae, which were then identified morphologically as *Ae. aegypti* using standard taxonomic keys.

Upon arrival at the IHMT, the mosquito eggs were reared under controlled insectary conditions, maintaining an environment of 27°C ± 1°C, with 60-70% relative humidity, and a 12:12 light-dark cycle to mimic natural conditions and ensure optimal development. Adult mosquitoes were provided with a blood meal two to three times per week to facilitate egg production, which is essential for maintaining the colony. For nutrition on non-feeding days, a 10% sucrose solution was provided, ensuring the mosquitoes had a continuous energy source.

These carefully controlled rearing conditions were crucial for maintaining the health and reproductive viability of the mosquito colony, which was used in subsequent experiments to assess vector competence for WNV.

2.4. Mosquito infection

Approximately 100 *Ae. aegypti* mosquitoes of each colony, aged around five days, were selected for the experimental infections. The mosquitoes were placed into cups and transported to an ACL3 (Arthropod Containment Biosafety Level 3) laboratory, where they were housed in an incubator (Percival Scientific Inc.) set to 27°C, with 70% relative humidity and a 12:12 light-dark cycle. The mosquitoes were kept unfed for 24 hours to enhance feeding behavior.

After the fasting period, the mosquitoes were offered a blood meal mixed with WNV in a 1:1 ratio. Specifically, 500 µl of defibrinated blood was mixed with 500 µl of the virus, which had an initial concentration

of 2 × 10^7 PFU/ml. The mixture was provided to the mosquitoes using a Hemotek feeding system, which held the virus-blood mixture in a 1 ml reservoir. The mosquitoes were allowed to feed for 30 minutes (Figure 9).

Following the feeding period, engorged females were separated from non-engorged females. The fed females were divided into groups of approximately 25 mosquitoes per cup and returned to the incubator. These mosquitoes were provided with a 10% sucrose solution throughout the remainder of the experiment. Mosquito mortality rates were recorded daily.

At three time points: 7-, 14-, and 21-days post-infection (dpi), 20 mosquitoes were dissected. Prior to dissection, the mosquitoes were anesthetized with CO_2 for 90 seconds. The wings and legs were removed, and the mosquitoes were induced to salivate by placing them in micropipette tips containing 10 µl of FBS for 30 minutes (Figure 9).

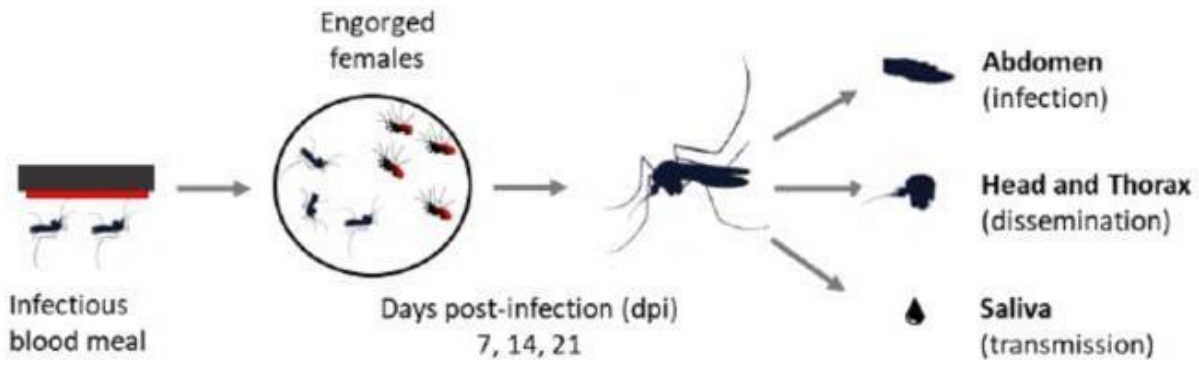

Figure 9. Infection and dissection method used. Source: (Ramírez et al., (2024).

Following the salivation period, the mosquitoes were dissected into two parts: the abdomen (body) and thorax together with the head. Each part was separated and placed into individual 2 ml tubes containing 300 µl of 1x DMEM supplemented with 10% FBS and 1% Pen/Strep. The micropipette tips containing FBS with collected saliva were then emptied into 0.2 ml centrifuge tubes pre-filled with 90 µl of 1x DMEM supplemented with 1% Pen/Strep. All tubes were promptly stored at -80°C for subsequent analysis.

The mosquito heads and bodies were then individually homogenized using a Precellys Evolution Homogenizer (Bertin Technologies). Approximately five 2 mm glass beads were added to each tube, and the samples were processed in two cycles at 2000 rpm for 20 seconds each. After homogenization, the samples were centrifuged at 5000g for 5 minutes to clarify the supernatant.

2.5. Molecular Biology

2.5.1. RNA extraction

RNA extraction was performed using NZYol reagent following the protocol provided by NZYtech for total RNA extraction.

After the mosquito tissues were homogenized, 100 µl of the homogenate was transferred to a separate 1.5 ml microcentrifuge tube. To this, 300 µl of NZYol was added, and the mixture was incubated for five minutes at room temperature to allow for the thorough lysis of the cells. Next, 60 µl of chloroform was added to the mixture, which was then rocked vigorously for 15 seconds to ensure proper mixing. The mixture was incubated for an additional three minutes at room temperature before being centrifuged at 12,000g for 15 minutes at 4°C. This step allowed for phase separation, with the aqueous phase containing the RNA. The aqueous (transparent) phase was carefully transferred to a new microcentrifuge tube, to which 150 µl of isopropyl alcohol was added to precipitate the RNA. The tube was incubated for 10 minutes at -20°C and then centrifuged at 12,000g for 10 minutes at 4°C. Following centrifugation, the supernatant was gently removed using a micropipette, leaving the RNA pellet at the bottom of the tube. The RNA pellet was washed by resuspension in 300 µl of 70% ethanol, followed by immediate centrifugation at 12,000g for five minutes at 4°C. After discarding the ethanol supernatant using a micropipette, the RNA pellet was air-dried for 10 minutes at room temperature to remove any residual ethanol. Once dried, the RNA pellet was resuspended in 30 µl of RNase-free water to preserve its integrity and then stored at -20°C until further analysis. For samples derived from mosquito saliva, the volumes used in the extraction process were halved, with the final RNA resuspension carried out in 20 µl of RNase-free water.

2.5.2. Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR)

The presence of WNV RNA was detected using a one-step Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR) assay. This method allows for the simultaneous conversion of RNA to cDNA and the amplification of specific viral sequences in a single reaction.

The primers and probe used in this assay were originally described by (Lanciotti et al., (2000) and are specifically designed to target the WNV genome. The RT-qPCR reaction was performed in a total volume of 10 µl, containing 5 µl of One-step NZYSupreme RT-qPCR Probe master mix, 0.4 µl of each primer of an initial concentration of 10µM, 0.1 µl of probe of an initial concentration of 10µM, 2.1 µl of DEPC-treated water and 2 µl of the RNA sample. The cycling conditions were 1 cycle of 20 min at 50 °C for cDNA synthesis, 1 cycle of 5 min at 95°C, 40 cycles of 5 seconds at 95°C followed by 30 seconds of 60°C.

Quantification of WNV RNA was achieved using a standard curve generated from serial dilutions of a known concentration of WNV RNA from a viral stock quantified in PFU/ml. This standard curve allowed for the precise determination of viral RNA concentrations in the samples, ensuring accurate and reproducible quantification.

2.6. Data analysis

The infection rate (IR), dissemination rate (DR), and transmission rate (TR) were calculated as follows:

- **Infection rate (IR)**: The proportion of mosquitoes with WNV-positive bodies out of the total number tested.
- **Dissemination rate (DR)**: The proportion of mosquitoes with WNV-positive legs out of the total number of infected mosquitoes.
- **Transmission rate (TR)**: The proportion of mosquitoes with WNV-positive saliva out of the total number of mosquitoes with disseminated infection.
- **Transmission efficiency (TE)**: proportion mosquitoes with WNV-positive saliva out of the total number of mosquitoes tested.

Statistical analysis was performed using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA). Differences in IR, DR, and TR and viral titers among different time points were assessed using Kruskal-Wallis test. A p-value of <0.05 was considered statistically significant.

3. Results

3.1. Mortality rates after experimental infection.

The mortality rates for the *Ae. aegypti* Rockefeller strain post-infection with WNV revealed minimal mortality across the experimental period (Table 1). Out of five cups, there was only death recorded in Cup 4, with two mosquitoes dying on day 12 post-infection. Dissections were performed on days 7, 14, and 21 post-infection, with 20 mosquitoes analyzed in each time point. Most of the Rockefeller strain mosquitoes of the initial 115 remained alive and were used for subsequent analysis, suggesting a high survival rate in this population under the experimental conditions used.

Table 1. Mortality rates for *Ae. aegypti* Rockefeller strain post-infection.

Day post-infection	Cup 1 (25♀)	Cup 2 (25♀)	Cup 3 (11♀)	Cup 4 (24♀)	Cup 5 (30♀)
1	0	0	0	0	0
2	0	0	0	0	0
5	0	0	0	0	0
6	0	0	0	0	0
7	mosquitoes dissected	0	0	0	0
8	-	0	0	0	0
9	-	0	0	0	0
12	-	0	0	2	0
13	-	0	0	0	0
14	-	0	0	0	mosquitoes dissected
15	-	0	0	0	-
16	-	0	0	0	-
19	-	0	0	0	-
20	-	0	0	0	-
21	-	mosquitoes dissected	mosquitoes dissected	mosquitoes dissected	-

The *Ae. aegypti* population from STP (Table 2) exhibited slightly higher mortality compared to the Rockefeller strain. Initially there were 77 mosquitoes, mortality was observed in Cup 3 (3 deaths on Day 3 post-infection), Cup 6 (2 deaths on Day 2 post-infection), Cup 7 (1 death on Day 2 post-infection), and Cup 8 (1 death on Day 10 post-infection). Dissections were conducted on Days 7, 14, and 21 post-infection, with 20 mosquitoes analyzed on each day. Despite these early mortalities, most of the population remained viable for subsequent analysis.

Table 2. Mortality rates for *Ae. aegypti* from STP post-infection.

Day Post-Infection	Cup 1 (7♀)	Cup 2 (5♀)	Cup 3 (8♀)	Cup 4 (3♀)	Cup 5 (2♀)	Cup 6 (10♀)	Cup 7 (22♀)	Cup 8 (16♀)	Cup 9 (4♀)
1	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	2	1	0	0
3	0	0	3	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0
7	0	mosquitoes dissected	mosquitoes dissected	0	0	mosquitoes dissected	0	0	mosquitoes dissected
8	0	-	-	0	0	-	0	0	-
9	0	-	-	0	0	-	0	0	-
10	0	-	-	0	0	-	0	1	-
11	0	-	-	0	0	-	0	0	-
12	0	-	-	0	0	-	0	0	-
13	0	-	-	0	0	-	0	0	-
14	mosquitoes dissected	-	-	mosquitoes dissected	0	-	0	mosquitoes dissected	-
15	-	-	-	-	0	-	0	-	-
16	-	-	-	-	0	-	0	-	-
17	-	-	-	-	0	-	0	-	-
18	-	-	-	-	0	-	0	-	-
19	-	-	-	-	0	-	0	-	-
20	-	-	-	-	0	-	0	-	-
21	-	-	-	-	mosquitoes dissected	-	mosquitoes dissected	-	-

3.2. Quantitative PCR (qPCR) analysis

The qPCR results demonstrated robust amplification, as evidenced by the well-defined standard curves generated during the analysis. All standard curves (an example in Figure 10) displayed a strong linear relationship between the logarithm of the starting quantity of the template and the cycle threshold (Cq) values, with an R^2 value always close to 1, indicating high precision and reliability of the assay. In the example of Figure 10, the slope of the curve was calculated to be -3.038, corresponding to an amplification efficiency of 113.4%, which is within acceptable limits for qPCR assays.

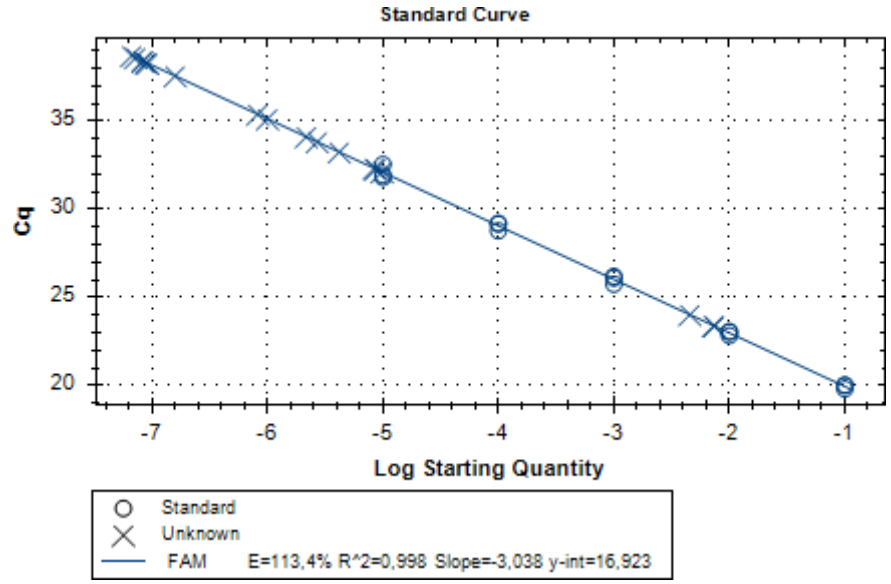

Figure 10. An example of a standard curve generated during qPCR analysis showing the relationship between the logarithm of the starting quantity of the template the stock was quantified using PFU/ml and the cycle threshold (Cq) values. Circles represent standard samples, while crosses denote unknown samples. E is the efficiency of the qPCR-how many amplicons are produced each cycle, R^2 is the determination coefficient-how good are the replicates, the slope also indicates efficiency, and the data is calibrated using y-intercept.

The amplification curves showed consistent and reproducible Cq values across different concentrations (Figure 11), confirming the efficiency of the primers and the accuracy of the qPCR setup. These results provide a reliable foundation for the subsequent analysis of WNV infection, dissemination, and transmission rates in *Ae. aegypti* populations.

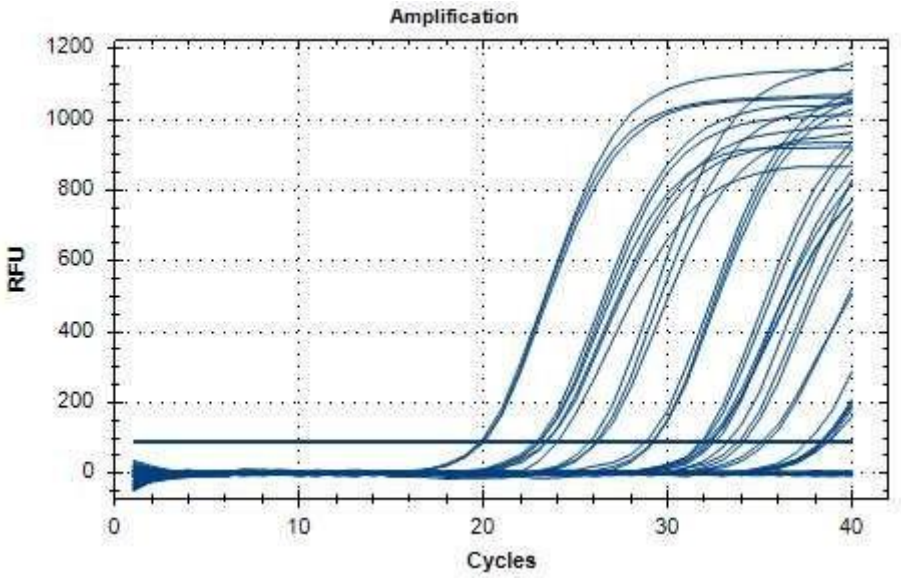

Figure 11. Amplification curves from the qPCR analysis. The curves represent real-time amplification of DNA across multiple samples, and that corresponds to multiple dilutions, indicating successful and consistent amplification. Each curve reflects the exponential increase in fluorescence as the target DNA is amplified during the qPCR cycles. The plot demonstrates the assay's ability to detect and quantify varying amounts of the target sequence.

To determine the viral load in experimental samples, standard curves were generated using serial dilutions of WNV RNA and the corresponding cycle threshold (Ct) values. The linear regression analysis of the standard curves always yielded a strong correlation (R^2 between 0.993 and 1), confirming the accuracy of the quantification method (Figure 12). The slope of the curve allowed for the precise calculation of viral RNA concentrations in the experimental samples, facilitating the assessment of infection, dissemination, and transmission rates and transmission efficiency in the mosquito populations tested.

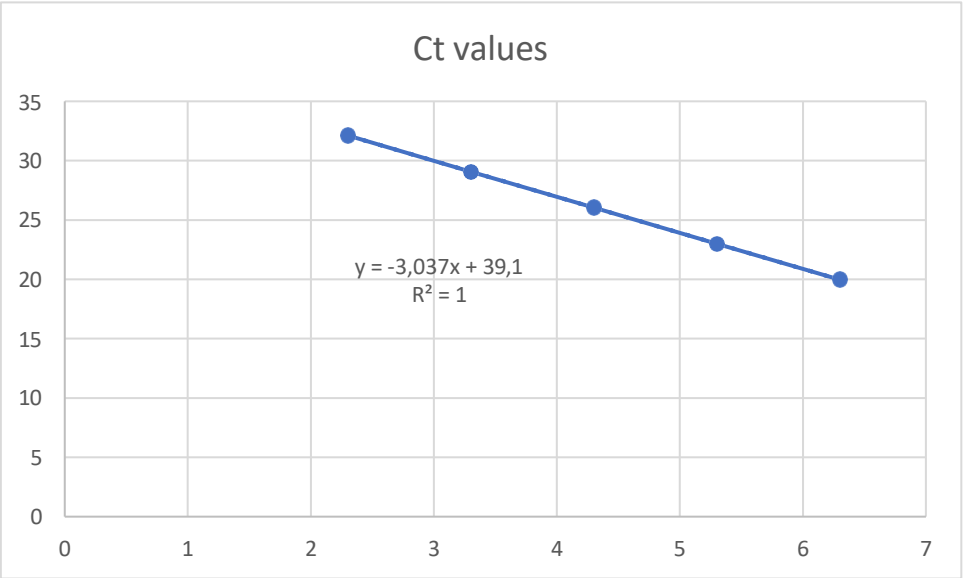

Figure 12. Example of a standard curve for viral RNA quantification generated from serial dilutions of WNV RNA after qPCR analysis. The linear regression equation describes the relationship between the log of RNA concentration and Cq values, with an R^2 value of 1, indicating a perfect fit and high accuracy in the quantification of viral RNA across the dilution series. This curve was used to determine the viral load in experimental samples.

3.3. Experimental infections

The infection, dissemination, transmission rates, and transmission efficiency of *Ae. aegypti* populations from STP and the Rockefeller strain exposed to the WNV PT6.39 strain were assessed over 21 dpi. Results are shown in Table 3 and 4.

Table 3. Infection, dissemination, transmission rates, and transmission efficiency for the population of *Ae. aegypti* from São Tomé and Príncipe exposed to WNV PT6.39 strain.

Days post infection	STP			
	IR % (n)	**DR % (n)**	**TR % (n)**	**TE % (n)**
7	5 (20)	0 (1)	0 (0)	0 (20)
14	20 (20)	100 (4)	25 (4)	5 (20)
21	35 (20)	43 (7)	67 (3)	10 (20)

Table 4. Infection, dissemination, transmission rates, and transmission efficiency for the population of *Ae. aegypti* Rockefeller exposed to WNV PT6.39 strain.

Days post infection	Rockefeller			
	IR % (n)	DR % (n)	TR % (n)	TE % (n)
7	0 (20)	0	0	0
14	0 (20)	0	0	0
21	0 (20)	0	0	0

Notes: Infection rate - percentage of WNV-positive bodies out of the total exposed mosquitoes; dissemination rate- percentage of virus-positive heads out of the total number of positive mosquito bodies; transmission efficiency- proportion of mosquitoes with positive saliva among the total number of fed mosquitoes; transmission rate- percentage of mosquitoes with infected saliva out of those with disseminated infection.

For the STP population, the infection rate (IR) increased over time, starting from 5% at 7 dpi to 35% at 21 dpi. The dissemination rate (DR) reached 100% at 14 dpi but dropped to 43% at 21 dpi. The transmission rate (TR) also increased from 0% at 7 dpi to 67% at 21 dpi. Transmission efficiency (TE) peaked at 10% by 21 dpi. The log10 viral titers in the body, head, and saliva of infected *Ae. aegypti* were measured at 7, 14, and 21 dpi (Figure 1). The mean viral titers were generally low across all tissues and time points, with the highest mean titer observed at 14 dpi. The standard deviation was relatively high at 14 and 21 dpi, indicating variability in viral replication among individual mosquitoes (Figure 13).

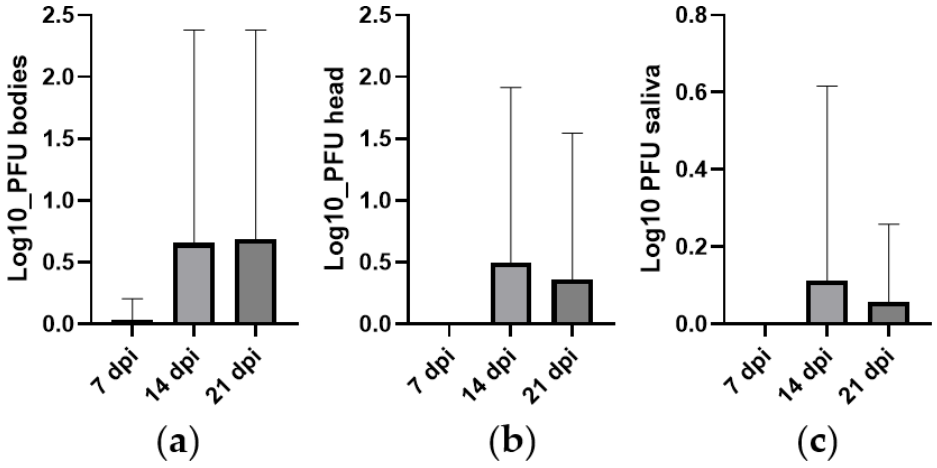

Figure 13. Number of viral particles in the abdomen (a), head/thorax (b) and saliva (c) *of Ae. aegypti* from STP exposed to WNV at 7, 14 and 21 dpi. Viral loads are presented as Log10 PFU/mL equivalents, which were calculated using standard curves generated from serial dilutions of WNV RNA and corresponding Ct values. Error bars represent the standard deviation.

The mean viral titer in the bodies increased slightly over time, with a mean of 0.037 log10 PFU/ml at 7 dpi, 0.656 log 10 PFU/ml at 14 dpi, and 0.689 log 10 PFU/ml at 21 dpi. However, the Kruskal-Wallis test showed no statistically significant difference in viral titers across the different time points ($\chi^2 = 5.36$, df = 2, P = 0.069).

Similar to the bodies, viral titers in the heads remained low across all time points, with a mean of 0.492 at 14 dpi and 0.359 at 21 dpi. The Kruskal-Wallis test also showed no significant differences across time points (χ^2 = 4.15, df = 2, P = 0.126). Viral titers in saliva were mostly low, with a mean of 0.112 log 10 PFU/ml at 14 dpi and 0.056 log 10 PFU/ml at 21 dpi. The Kruskal-Wallis test indicated no significant differences across the time points (χ^2 = 2.00, df = 2, P = 0.368). Although viral titers varied slightly between different tissues and time points, no significant differences were detected overall. This suggests that while some mosquitoes did develop higher viral loads, the overall replication and transmission potential of WNV in the *Ae. aegypti* population from STP remained relatively low.

The *Ae. aegypti* Rockefeller strain consistently showed no evidence of WNV infection, dissemination, or transmission across all time points (7-, 14-, and 21 dpi).

4. Discussion and Conclusions

This study offers crucial insights into the vector competence of *Ae. aegypti* from STP for WNV transmission. Despite WNV not being documented in STP, our proactive approach aims to assess the potential risk if the virus was introduced, possibly via migratory birds, increased globalization or other vectors. Conducting such studies in regions without current WNV presence is essential for predicting and preparing for possible outbreaks.

Our findings indicate that while *Ae. aegypti* from STP can become infected with WNV, the overall transmission potential remains low. The infection rate gradually increased from 5% at 7 dpi to 35% at 21 dpi, showing susceptibility to WNV, though to a lesser extent than *Culex* species, known as more competent WNV vectors(Turell et al., 2001). This reduced susceptibility may be due to genetic differences, environmental factors, or the specific viral strain used. Dissemination rates peaked at 100% by 14 dpi but decreased to 43% by 21 dpi, possibly reflecting reduced viral replication or an effective immune response within the mosquitoes. Transmission rates and efficiency, although increasing over time, remained low overall, suggesting that even when the virus successfully disseminates within the mosquito, only a small proportion of the latter can transmit the virus effectively. This finding aligns with previous research indicating that *Ae. aegypti* is not a highly competent vector for WNV (Turell et al., 2001), which contrasts with its role as a primary vector for other arboviruses like dengue and Zika. The barriers that limit viral replication and dissemination to the salivary glands in *Ae. aegypti* are likely significant factors contributing to the observed low transmission efficiency, the barrier of the salivary gland can stop viral entry into the salivary glands, so it is a barrier that has been studied recently (Sanchez-Vargas et al., 2021). These barriers may include physiological defenses within the mosquito that prevent the virus from reaching the salivary glands or from replicating efficiently once there.

The Rockefeller strain's complete lack of WNV infection, dissemination, or transmission may be due to either the strain's apparent resistance, or very low susceptibility, to the WNV PT6.39 strain with the viral dose used (approximately 10^7 PFU/ml) (which contrasts sharply with the *Ae. aegypti* population from STP), a specific resistance or a loss of vector competence due to extensive inbreeding and adaptation to laboratory conditions or even an experimental error due to the fact that the infections of the *Ae. aegypti* of STP and Rockefeller strain were not made in the same day. This issue, observed in *Aedes aegypti* colonies reared under low population sizes, can lead to fitness costs (Ross, Endersby-Harshman, et al., 2019). Long-term laboratory-rearing conditions can alter the genetic diversity of mosquito populations, causing them to drift genetically from their wild counterparts. For instance, *Ae. aegypti* laboratory strains have shown reduced genetic variation due to bottleneck effects and adaptation to artificial rearing conditions. This can significantly impact

traits like vector competence and fitness, as these lab strains no longer reflect the genetic diversity or environmental pressures found in wild populations (Dickson et al., 2018); (Gloria-Soria et al., 2019).

Given the findings of this study, future research should explore several avenues to expand our knowledge of the vector competence of *Ae. aegypti* and other mosquito species from STP for WNV. First, the use of higher WNV viral doses might provide insights into whether the barriers observed at lower doses can be overcome. Increased viral loads may lead to more robust infections and could provide insights into the thresholds required for effective WNV transmission. Additionally, it is important to examine the vector competence of *Ae. aegypti* for other WNV lineages, particularly lineage 2, which is known to circulate in regions near STP and may exhibit different infectivity and transmission patterns (Mencatelli et al., 2022). Lineage 2 is increasingly prevalent in Europe and Africa, making it a critical focus for vector competence studies, especially in regions like STP, where its introduction could alter the epidemiological landscape of WNV transmission.

Expanding the scope of research to include other mosquito species, such as *Ae. albopictus* and *Cx. quinquefasciatus*, would provide a more comprehensive understanding of the potential for WNV transmission in STP. *Culex* species, in particular, are recognized as the primary vectors of WNV globally, and their presence in STP could play a significant role in sustaining WNV cycles if the virus is introduced. The inclusion of *Ae. albopictus* is also essential, as this species is known to be a secondary vector for WNV in several regions and has shown adaptability to urban environments, increasing its potential as a bridge vector between avian and human populations (Rothman et al., 2021); (Martinet et al., 2023).

Moreover, the use of animal models to study WNV and mosquito interactions represents another promising avenue for future research. A recent study by (Baldon et al., (2024) demonstrated the utility of an immunocompromised AG129 mouse model for studying WNV in *Cx. quinquefasciatus*. Applying similar models to assess the interactions of WNV with mosquitoes present in STP could yield insights relevant to local transmission dynamics. Finally, studies that compare genetic differences between mosquito populations in terms of WNV susceptibility could shed light on the mechanisms influencing vector competence. Genetic factors play a crucial role in determining vector competence, as certain genes (Hk2, Got-1) can enhance or inhibit viral replication and dissemination (Failloux et al., 2002); (Beerntsen et al., 2000). Identifying these genes and understanding their role in WNV transmission could help guide targeted vector control strategies.

Some limitations of this study include the relatively small sample size of mosquitoes dissected and analyzed at each time point, though it is important to note that using 20 mosquitoes per sampling time point is standard practice in vector competence studies. This number provides a statistically valid representation for observing infection, dissemination, and transmission trends. However, the generalizability of the findings could still benefit from larger sample sizes in future studies to confirm the robustness of the results. Additionally, the mosquitoes were reared under controlled laboratory conditions, which, while useful for

controlling variables, may not fully replicate the complexities of natural settings. Factors such as temperature fluctuations, food availability, and natural exposure to pathogens could impact the vector competence of mosquitoes differently in the field (Alto & Lounibos, 2013), the microbiota also affects vector competence (figure 7). Furthermore, the observed variability in infection, dissemination, and transmission rates across different mosquito populations suggests underlying genetic differences influencing vector competence. While this was not specifically studied in this thesis, understanding the genetic basis for such differences would offer valuable insights into the mechanisms driving vector competence. Future studies could incorporate genetic analysis to explore how variations in mosquito genotypes contribute to differences in WNV susceptibility and transmission potential, particularly in the context of evolving mosquito populations and viral strains.

Given the complex interactions between environmental factors, mosquito species, and viral strains, continuous surveillance is crucial. Control measures should be tailored to the specific environmental conditions, mosquito species present, their behavior, and other local factors in a particular area. For example, knowing which mosquito species are prevalent, their breeding sites, and how they interact with humans can help design more effective interventions, such as targeted insecticide application, habitat management, or community-based control efforts that address the local risk factors for WNV transmission. In STP, preventive strategies should focus on reducing the risk of WNV introduction. This can be achieved by monitoring and controlling mosquito populations known to be competent vectors for WNV and conducting surveillance of potential reservoirs, such as migratory birds, that could introduce the virus into the region.

In conclusion, while *Ae. aegypti* from STP demonstrated the ability to be infected with WNV, their overall vector competence remains limited. This study contributes to the growing body of research on mosquito-borne arboviruses in Africa and highlights the need for region-specific studies to inform vector control strategies. The limited transmission efficiency observed in *Ae. aegypti* under the experimental conditions used suggests that, although capable of acquiring and disseminating the virus, the species is not an efficient vector for WNV compared to primary vectors like *Culex* species. By enhancing our understanding of vector competence and transmission dynamics, public health authorities will be better equipped to design preventative measures and respond to potential outbreaks, thereby protecting communities from the spread of WNV and similar arboviruses.

5. References

Agarwal, A., Parida, M., & Dash, P. K. (2017). Impact of transmission cycles and vector competence on global expansion and emergence of arboviruses. In Reviews in Medical Virology (Vol. 27, Issue 5). John Wiley and Sons Ltd. https://doi.org/10.1002/rmv.1941

Almeida, A. P., Gonçalves, Y. M., Novo, M. T., Sousa, C. A., Melim, M., & Gracio, A. J. (2007). Vector monitoring of *Aedes aegypti* in the Autonomous Region of Madeira, Portugal. Weekly Releases (1997–2007), 12(46). https://doi.org/10.2807/esw.12.46.03311-en

Alto, B. W., & Lounibos, L. P. (2013). Vector competence for arboviruses in relation to the larval environment of mosquitoes. In Ecology of parasite-vector interactions (pp. 81–101). Wageningen Academic Publishers. https://doi.org/10.3920/978-90-8686-744-8_4

Baldon, L., de Mendonça, S., Santos, E., Marçal, B., de Freitas, A. C., Rezende, F., Moreira, R., Sousa, V., Comini, S., Lima, M., Ferreira, F., de Almeida, J. P., Silva, E., Amadou, S., Rocha, M., Leite, T., Todjro, Y., de Carvalho, C., Santos, V., … Ferreira, A. (2024). Suitable Mouse Model to Study Dynamics of West Nile Virus Infection in *Culex quinquefasciatus* Mosquitoes. Tropical Medicine and Infectious Disease, 9(9), 201. https://doi.org/10.3390/tropicalmed9090201

Bartholomay, L. C., Waterhouse, R. M., Mayhew, G. F., Campbell, C. L., Michel, K., Zou, Z., Ramirez, J. L., Das, S., Alvarez, K., Arensburger, P., Bryant, B., Chapman, S. B., Dong, Y., Erickson, S. M., Parakrama Karunaratne, S. H. P., Kokoza, V., Kodira, C. D., Pignatelli, P., Shin, S. W., … Muskavitch, M. A. T. (2010). Pathogenomics of *Culex quinquefasciatus* and meta-analysis of infection responses to diverse pathogens. Science, 330(6000), 88–90. https://doi.org/10.1126/science.1193162

Beerntsen, B. T., James, A. A., & Christensen, B. M. (2000). Genetics of Mosquito Vector Competence. In MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS (Vol. 64, Issue 1). https://journals.asm.org/journal/mmbr

BEI Reagent Search. (n.d.). Retrieved September 1, 2024, from https://www.beiresources.org/Catalog/BEIVectors/MRA-734.aspx

BirdLife International (BirdLife International). (2024, July 4). IUCN Red List of Threatened Species: Numenius phaeopus. IUCN Red List of Threatened Species. Retrieved February 16, 2025, from https://www.iucnredlist.org/species/22693178/138413707

Bohers, C., Vazeille, M., Bernaoui, L., Pascalin, L., Meignan, K., Mousson, L., Jakerian, G., Karch, A., de Lamballerie, X., & Failloux, A. B. (2024). *Aedes albopictus* is a competent vector of five arboviruses affecting human health, greater Paris, France, 2023. Eurosurveillance, 29(20). https://doi.org/10.2807/1560-7917.ES.2024.29.20.2400271

Bonizzoni, M., Dunn, W. A., Campbell, C. L., Olson, K. E., Marinotti, O., & James, A. A. (2012). Complex Modulation of the *Aedes aegypti* Transcriptome in Response to Dengue Virus Infection. PLoS ONE, 7(11). https://doi.org/10.1371/journal.pone.0050512

Brien, J. D., Lazear, H. M., & Diamond, M. S. (2013). Propagation, Quantification, Detection, and Storage of West Nile Virus. Current Protocols in Microbiology, 31(1). https://doi.org/10.1002/9780471729259.mc15d03s31

Brinton, M. A. (2013). Replication cycle and molecular biology of the west nile virus. Viruses, 6(1), 13–53. https://doi.org/10.3390/v6010013

Cansado-Utrilla, C., Zhao, S. Y., McCall, P. J., Coon, K. L., & Hughes, G. L. (2021). The microbiome and mosquito vectorial capacity: rich potential for discovery and translation. In Microbiome (Vol. 9, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s40168-021-01073-2

Ciota, A. T. (2017). West Nile virus and its vectors. In Current Opinion in Insect Science (Vol. 22, pp. 28–36). Elsevier Inc. https://doi.org/10.1016/j.cois.2017.05.002

Ciota, A. T., Ehrbar, D. J., Matacchiero, A. C., van Slyke, G. A., & Kramer, L. D. (2013). The evolution of

virulence of West Nile virus in a mosquito vector: Implications for arbovirus adaptation and evolution. BMC Evolutionary Biology, 13(1). https://doi.org/10.1186/1471-2148-13-71

Colpitts Editor, T. M. (n.d.-a). West Nile Virus Methods and Protocols Methods in Molecular Biology 1435. http://www.springer.com/series/7651

Colpitts, T. M., Conway, M. J., Montgomery, R. R., & Fikrig, E. (2012). West Nile virus: Biology, transmission, and human infection. Clinical Microbiology Reviews, 25(4), 635–648. https://doi.org/10.1128/CMR.00045-12

Current year data (2024). (2025, January 14). West Nile Virus. Retrieved August 30, 2024, from https://www.cdc.gov/west-nile-virus/data-maps/current-year-data.html

Danis, K., Papa, A., Theocharopoulos, G., Dougas, G., Athanasiou, M., Detsis, M., Baka, A., Lytras, T., Mellou, K., Bonovas, S., & Panagiotopoulos, T. (2011). Outbreak of West Nile virus infection in Greece, 2010. Emerging Infectious Diseases, 17(10), 1868–1872. https://doi.org/10.3201/eid1710.110525

de Lima, R. F., & Melo, M. (2021). A revised bird checklist for the oceanic islands of the Gulf of Guinea (Príncipe, São Tomé and Annobón). Bulletin of the British Ornithologists' Club, 141(2), 179–198. https://doi.org/10.25226/bboc.v141i2.2021.a9

de Oliveira, W. K., de França, G. V. A., Carmo, E. H., Duncan, B. B., de Souza Kuchenbecker, R., & Schmidt, M. I. (2017). Infection-related microcephaly after the 2015 and 2016 Zika virus outbreaks in Brazil: a surveillance-based analysis. The Lancet, 390(10097), 861–870. https://doi.org/10.1016/S0140-6736(17)31368-5

Department of Health. Victoria, Australia. (2025, January 7). West Nile virus and West Nile virus/Kunjin disease. Retrieved September 30, 2024, from https://www.health.vic.gov.au/infectious-diseases/west-nile-virus-and-west-nile-virus-kunjin-disease

Depoortere, E., Kavle, J., Keus, K., Zeller, H., Murri, S., & Legros, D. (2004). Outbreak of West Nile virus causing severe neurological involvement in children, Nuba Mountains, Sudan, 2002. Tropical Medicine and International Health, 9(6), 730–736. https://doi.org/10.1111/j.1365-3156.2004.01253.x

Dickson, L. B., Ghozlane, A., Volant, S., Bouchier, C., Ma, L., Vega-Rúa, A., Dusfour, I., Jiolle, D., Paupy, C., Mayanja, M. N., Kohl, A., Lutwama, J. J., Duong, V., & Lambrechts, L. (2018). Diverse laboratory colonies of *Aedes aegypti* harbor the same adult midgut bacterial microbiome. Parasites and Vectors, 11(1). https://doi.org/10.1186/s13071-018-2780-1

Dohm, D. J., O'Guinn, M. L., & Turell, M. J. (2002). Effect of environmental temperature on the ability of *Culex pipiens* (Diptera: *Culicidae*) to transmit West Nile virus. Journal of Medical Entomology, 39(1), 221–225. https://doi.org/10.1603/0022-2585-39.1.221

Esteves, A., Almeida, A. P. G., Galão, R. P., Parreira, R., Piedade, J., Rodrigues, J. C., Sousa, C. A., & Novo, M. T. (2005). West Nile Virus in Southern Portugal, 2004. Vector-Borne and Zoonotic Diseases, 5(4), 410–413. https://doi.org/10.1089/vbz.2005.5.410

Failloux, A. B., Vazeille, M., & Rodhain, F. (2002). Geographic genetic variation in populations of the dengue virus vector *Aedes aegypti*. Journal of Molecular Evolution, 55(6), 653–663. https://doi.org/10.1007/s00239-002-2360-y

Fall, G., Diallo, M., Loucoubar, C., Faye, O., & Sall, A. A. (2014). Vector competence of *Culex neavei* and *Culex quinquefasciatus* (Diptera: *Culicidae*) from Senegal for lineages 1, 2, Koutango and a putative new lineage of West Nile virus. American Journal of Tropical Medicine and Hygiene, 90(4), 747–754. https://doi.org/10.4269/ajtmh.13-0405

Foil, L. D., & Gorham, J. R. (2000). Mechanical Transmission of Disease Agents by Arthropods. In Medical Entomology (pp. 461–514). Springer Netherlands. https://doi.org/10.1007/978-94-011-6472-6_12

García-Carrasco, J. M., Muñoz, A. R., Olivero, J., Figuerola, J., Fa, J. E., & Real, R. (2023). Gone (and spread) with the birds: Can chorotype analysis highlight the spread of West Nile virus within the Afro-

Palaearctic flyway? One Health, 17. https://doi.org/10.1016/j.onehlt.2023.100585

Gil-Santana, H. R., Brockmann, E., & Alencar, J. (2021). The correct pronunciation of the generic name *Aedes*, to which *Aedes aegypti* belongs. Anais Da Academia Brasileira de Ciencias, 93(3). https://doi.org/10.1590/0001-3765202120201012

Gloria-Soria, A., Soghigian, J., Kellner, D., & Powell, J. R. (2019). Genetic diversity of laboratory strains and implications for research: The case of *Aedes aegypti*. PLoS Neglected Tropical Diseases, 13(12). https://doi.org/10.1371/JOURNAL.PNTD.0007930

Habarugira, G., Suen, W. W., Hobson-Peters, J., Hall, R. A., & Bielefeldt-Ohmann, H. (2020). West nile virus: An update on pathobiology, epidemiology, diagnostics, control and "One health" implications. In Pathogens (Vol. 9, Issue 7, pp. 1–51). MDPI AG. https://doi.org/10.3390/pathogens9070589

Halasa, Y. A., Shepard, D. S., & Zeng, W. (2012). Economic cost of dengue in Puerto Rico. American Journal of Tropical Medicine and Hygiene, 86(5), 745–752. https://doi.org/10.4269/ajtmh.2012.11-0784

Hanna, S. L., Pierson, T. C., Sanchez, M. D., Ahmed, A. A., Murtadha, M. M., & Doms, R. W. (2005). N-Linked Glycosylation of West Nile Virus Envelope Proteins Influences Particle Assembly and Infectivity. Journal of Virology, 79(21), 13262–13274. https://doi.org/10.1128/jvi.79.21.13262-13274.2005

Hardy, J. L., Houk, E. J., Kramer, L. D., & Reeves, W. C. (1983). Intrinsic Factors Affecting Vector Competence of Mosquitoes for Arboviruses. Annual Review of Entomology, 28(1), 229–262. https://doi.org/10.1146/annurev.en.28.010183.001305

HAYES, C. G. (2001). West Nile Virus: Uganda, 1937, to New York City, 1999. Annals of the New York Academy of Sciences, 951(1), 25–37. https://doi.org/10.1111/j.1749-6632.2001.tb02682.x

Heitmann, A., Jansen, S., Lühken, R., Leggewie, M., Schmidt-Chanasit, J., & Tannich, E. (2018). Forced salivation as a method to analyze vector competence of mosquitoes. Journal of Visualized Experiments, 2018(138). https://doi.org/10.3791/57980

Historic data (1999-2023). (2024, June 18). West Nile Virus. Retrieved September 30, 2024, from https://www.cdc.gov/west-nile-virus/data-maps/historic-data.html

Hugo, L. E., Rašić, G., Maynard, A. J., Ambrose, L., Liddington, C., Thomas, C. J. E., Nath, N. S., Graham, M., Winterford, C., Randika Wimalasiri-Yapa, B. M. C., Xi, Z., Beebe, N. W., & Devine, G. J. (2022). Wolbachia wAlbB inhibit dengue and Zika infection in the mosquito *Aedes aegypti* with an Australian background. PLoS Neglected Tropical Diseases, 16(10). https://doi.org/10.1371/journal.pntd.0010786

Iunicheva, I. v, Riabova, T. E., Markovich, N. I., Bezzhonova, O. v, Ganushkina, L. A., Semenov, V. B., Tarkhov, G. A., Vasilenko, L. E., Guzeeva, T. M., Shevereva, T. v, & Sergiev, V. P. (2008). [First evidence for breeding *Aedes aegypti* L in the area of Greater Sochi and in some towns of Abkhasia]. Meditsinskaia Parazitologiia i Parazitarnye Bolezni, 3, 40–43.

Jennings, C. D., & Kay, B. H. (1999). Dissemination barriers to Ross River virus in *Aedes vigilax* and the effects of larval nutrition on their expression. Medical and Veterinary Entomology, 13(4), 431–438. https://doi.org/10.1046/j.1365-2915.1999.00196.x

Kain, M. P., Skinner, E. B., Athni, T. S., Ramirez, A. L., Mordecai, E. A., & van den Hurk, A. F. (2022). Not all mosquitoes are created equal: A synthesis of vector competence experiments reinforces virus associations of Australian mosquitoes. PLoS Neglected Tropical Diseases, 16(10). https://doi.org/10.1371/journal.pntd.0010768

Kamgang, B., Acântara, J., Tedjou, A., Keumeni, C., Yougang, A., Ancia, A., Bigirimana, F., Clarke, S. E., Gil, V. S., & Wondji, C. (2024). Entomological surveys and insecticide susceptibility profile of *Aedes aegypti* during the dengue outbreak in Sao Tome and Principe in 2022. PLoS Neglected Tropical Diseases, 18(6). https://doi.org/10.1371/journal.pntd.0011903

Karim, S. U., & Bai, F. (2023). Introduction to West Nile Virus. In Methods in Molecular Biology (Vol. 2585, pp. 1–7). Humana Press Inc. https://doi.org/10.1007/978-1-0716-2760-0_1

Khare, B., & Kuhn, R. J. (2022). The Japanese Encephalitis Antigenic Complex Viruses: From Structure to Immunity. In Viruses (Vol. 14, Issue 10). MDPI. https://doi.org/10.3390/v14102213

Kilpatrick, A. M., Fonseca, D. M., Ebel, G. D., Reddy, M. R., & Kramer, L. D. (2010). Spatial and Temporal Variation in Vector Competence of *Culex pipiens* and *Cx. restuans* Mosquitoes for West Nile Virus. The American Society of Tropical Medicine and Hygiene, 83(3), 607–613. https://doi.org/10.4269/ajtmh.2010.10-0005

Lanciotti, R. S., Kerst, A. J., Nasci, R. S., Godsey, M. S., Mitchell, C. J., Savage, H. M., Komar, N., Panella, N. A., Allen, B. C., Volpe, K. E., Davis, B. S., & Roehrig, J. T. (2000). Rapid Detection of West Nile Virus from Human Clinical Specimens, Field-Collected Mosquitoes, and Avian Samples by a TaqMan Reverse Transcriptase-PCR Assay. In JOURNAL OF CLINICAL MICROBIOLOGY (Vol. 38, Issue 11).

Lewis, J., Gallichotte, E. N., Randall, J., Glass, A., Foy, B. D., Ebel, G. D., & Kading, R. C. (2023). Intrinsic factors driving mosquito vector competence and viral evolution: a review. In Frontiers in Cellular and Infection Microbiology (Vol. 13). Frontiers Media SA. https://doi.org/10.3389/fcimb.2023.1330600

Life cycle of *Aedes* mosquitoes. (2024, April 16). Mosquitoes. Retrieved April 16, 2024, from https://www.cdc.gov/mosquitoes/about/life-cycle-of-*Aedes*-mosquitoes.html

Lim, S. M., Koraka, P., Osterhaus, A. D. M. E., & Martina, B. E. E. (2011). West Nile virus: Immunity and pathogenesis. In Viruses (Vol. 3, Issue 6, pp. 811–828). https://doi.org/10.3390/v3060811

Lindenbach, B. D., & Rice, C. M. (2003). Molecular biology of flaviviruses (pp. 23–61). https://doi.org/10.1016/S0065-3527(03)59002-9

Linthout, C., Martins, A. D., de Wit, M., Delecroix, C., Abbo, S. R., Pijlman, G. P., & Koenraadt, C. J. M. (2024). The potential role of the Asian bush mosquito *Aedes japonicus* as spillover vector for West Nile virus in the Netherlands. Parasites and Vectors, 17(1). https://doi.org/10.1186/s13071-024-06279-5

Liu, Z. Y., Shi, W. F., & Qin, C. F. (2019). The evolution of Zika virus from Asia to the Americas. In Nature Reviews Microbiology (Vol. 17, Issue 3, pp. 131–139). Nature Publishing Group. https://doi.org/10.1038/s41579-018-0134-9

Londono-Renteria, B., & Colpitts, T. M. (2016). A brief review of West Nile virus biology. In Methods in Molecular Biology (Vol. 1435). Humana Press Inc. https://doi.org/10.1007/978-1-4939-3670-0_1

Lu, L., Zhang, F., Munnink, B. B. O., Munger, E., Sikkema, R. S., Pappa, S., Tsioka, K., Sinigaglia, A., Dal Molin, E., Shih, B. B., Günther, A., Pohlmann, A., Ziegler, U., Beer, M., Taylor, R. A., Bartumeus, F., Woolhouse, M., Aarestrup, F. M., Barzon, L., … Koopmans, M. P. G. (2024). West Nile virus spread in Europe: Phylogeographic pattern analysis and key drivers. PLoS Pathogens, 20(1). https://doi.org/10.1371/journal.ppat.1011880

Lupia, T., Libanore, V., Corcione, S., Fornari, V., Rizzello, B., Bosio, R., Stroffolini, G., Bigliano, P., Fontana, S., Patti, F., Brusa, M. T., Degioanni, M., Concialdi, E., Navazio, A. S., Penna, M., & de Rosa, F. G. (2022). Autochthonous West Nile Virus Infection Outbreak in Humans (Asti, Piedmont, Italy, August–October 2018) and Long-Term Sequelae Follow-Up. Tropical Medicine and Infectious Disease, 7(8). https://doi.org/10.3390/tropicalmed7080185

Lwande, O. W., Obanda, V., Lindström, A., Ahlm, C., Evander, M., Näslund, J., & Bucht, G. (2020). Globe-Trotting *Aedes aegypti* and *Aedes albopictus*: Risk Factors for Arbovirus Pandemics. In Vector-Borne and Zoonotic Diseases (Vol. 20, Issue 2, pp. 71–81). Mary Ann Liebert Inc. https://doi.org/10.1089/vbz.2019.2486

Martinet, J. P., Bohers, C., Vazeille, M., Ferté, H., Mousson, L., Mathieu, B., Depaquit, J., & Failloux, A. B. (2023). Assessing vector competence of mosquitoes from northeastern France to West Nile virus and Usutu virus. PLoS Neglected Tropical Diseases, 17(6 June). https://doi.org/10.1371/journal.pntd.0011144

McAuley, A. J., & Beasley, D. W. C. (2016). Propagation and Titration of West Nile Virus on Vero Cells (pp.

19–27). https://doi.org/10.1007/978-1-4939-3670-0_3

McBride, C. S., Baier, F., Omondi, A. B., Spitzer, S. A., Lutomiah, J., Sang, R., Ignell, R., & Vosshall, L. B. (2014). Evolution of mosquito preference for humans linked to an odorant receptor. Nature, 515(7526), 222–227. https://doi.org/10.1038/nature13964

Medigeshi, G. R., Hirsch, A. J., Streblow, D. N., Nikolich-Zugich, J., & Nelson, J. A. (2008). West Nile Virus Entry Requires Cholesterol-Rich Membrane Microdomains and Is Independent of αvβ3 Integrin. Journal of Virology, 82(11), 5212–5219. https://doi.org/10.1128/jvi.00008-08

Mencattelli, G., Ndione, M. H. D., Rosà, R., Marini, G., Diagne, C. T., Diagne, M. M., Fall, G., Faye, O., Diallo, M., Faye, O., Savini, G., & Rizzoli, A. (2022). Epidemiology of West Nile virus in Africa: An underestimated threat. PLoS Neglected Tropical Diseases, 16(1). https://doi.org/10.1371/JOURNAL.PNTD.0010075

Mencattelli, G., Silverj, A., Iapaolo, F., Ippoliti, C., Teodori, L., di Gennaro, A., Curini, V., Candeloro, L., Conte, A., Polci, A., Morelli, D., Perrotta, M. G., Marini, G., Rosà, R., Monaco, F., Segata, N., Rizzoli, A., Rota-Stabelli, O., & Savini, G. (2023). Epidemiological and Evolutionary Analysis of West Nile Virus Lineage 2 in Italy. Viruses, 15(1). https://doi.org/10.3390/v15010035

Merwaiss, F., Filomatori, C. v, Susuki, Y., Bardossy, E. S., Alvarez, D. E., & Saleh, M.-C. (2021). Chikungunya Virus Replication Rate Determines the Capacity of Crossing Tissue Barriers in Mosquitoes. Journal of Virology, 2021(3), 1956–1976. https://doi.org/10.1128/JVI.01956-20ï

Moudy, R. M., Meola, M. A., Morin, L.-L. L., Ebel, G. D., & Kramer, L. D. (2017). A Newly Emergent Genotype of West Nile Virus Is Transmitted Earlier and More Efficiently by *Culex* Mosquitoes.

Mukhopadhyay, S., Kuhn, R. J., & Rossmann, M. G. (2005). A structural perspective of the Flavivirus life cycle. In Nature Reviews Microbiology (Vol. 3, Issue 1, pp. 13–22). https://doi.org/10.1038/nrmicro1067

Ndione, M. H. D., Ndiaye, E. H., Faye, M., Diagne, M. M., Diallo, D., Diallo, A., Sall, A. A., Loucoubar, C., Faye, O., Diallo, M., Faye, O., Barry, M. A., & Fall, G. (2022). Re-Introduction of West Nile Virus Lineage 1 in Senegal from Europe and Subsequent Circulation in Human and Mosquito Populations between 2012 and 2021. Viruses, 14(12). https://doi.org/10.3390/v14122720

Obradovic, Z., Smjecanin, E., Pindzo, E., Omerovic, H., & Cibo, N. (2022). A LITERATURE REVIEW ON VECTOR BORNE DISEASES. International Journal of Medical Reviews and Case Reports, 6(Reports in Surgery, Orthopaedi), 1. https://doi.org/10.5455/ijmrcr.172-1639404085

PAHO/WHO Data - National Dengue fever cases | PAHO/WHO. (n.d.). Pan American Health Organization / World Health Organization. Retrieved September 30, 2024, from https://www3.paho.org/data/index.php/en/mnu-topics/indicadores-dengue-en/dengue-nacional-en/252-dengue-pais-ano-en.html

Petersen, L. R., Brault, A. C., & Nasci, R. S. (2013). West Nile virus: Review of the literature. In JAMA (Vol. 310, Issue 3, pp. 308–315). American Medical Association. https://doi.org/10.1001/jama.2013.8042

Powell, J. R., Gloria-Soria, A., & Kotsakiozi, P. (2018). Recent history of *Aedes aegypti*: Vector genomics and epidemiology records. In BioScience (Vol. 68, Issue 11, pp. 854–860). Oxford University Press. https://doi.org/10.1093/biosci/biy119

Powell, J. R., & Tabachnick, W. J. (2013). History of domestication and spread of *Aedes aegypti*--a review. In Memórias do Instituto Oswaldo Cruz (Vol. 108, pp. 11–17). https://doi.org/10.1590/0074-0276130395

Rainey, S. M., Shah, P., Kohl, A., & Dietrich, I. (2014). Understanding the wolbachia-mediated inhibition of arboviruses in mosquitoes: Progress and challenges. In Journal of General Virology (Vol. 95, Issue PART3, pp. 517–530). https://doi.org/10.1099/vir.0.057422-0

Ramírez, R. M. G., Bohers, C., Mousson, L., Madec, Y., Vazeille, M., Piorkowski, G., Moutailler, S., Diaz, F. J., Rúa-Uribe, G., Villar, L. A., de Lamballerie, X., & Failloux, A. B. (2024). Increased threat of urban

arboviral diseases from *Aedes aegypti* mosquitoes in Colombia. IJID Regions, 11. https://doi.org/10.1016/j.ijregi.2024.100360

Ross, P. A., Endersby-Harshman, N. M., & Hoffmann, A. A. (2019). A comprehensive assessment of inbreeding and laboratory adaptation in *Aedes aegypti* mosquitoes. Evolutionary Applications, 12(3), 572–586. https://doi.org/10.1111/eva.12740

Ross, P. A., Endersby-Harshman, N. M., & Hoffmann, A. A. (2019). A comprehensive assessment of inbreeding and laboratory adaptation in *Aedes aegypti* mosquitoes. Evolutionary Applications, 12(3), 572–586. https://doi.org/10.1111/eva.12740

Rothman, S. E., Jones, J. A., Ladeau, S. L., & Leisnham, P. T. (2021). Higher West Nile Virus Infection *in Aedes albopictus* (Diptera: *Culicidae*) and *Culex* (Diptera: *Culicidae*) Mosquitoes from Lower Income Neighborhoods in Urban Baltimore, MD. Journal of Medical Entomology, 58(3), 1424–1428. https://doi.org/10.1093/jme/tjaa262

Sanchez-Vargas, I., Olson, K. E., & Black, W. C. (2021). The genetic basis for salivary gland barriers to arboviral transmission. Insects, 12(1), 1–22. https://doi.org/10.3390/insects12010073

Smith, D. L., Battle, K. E., Hay, S. I., Barker, C. M., Scott, T. W., & McKenzie, F. E. (2012). Ross, Macdonald, and a Theory for the Dynamics and Control of Mosquito-Transmitted Pathogens. PLoS Pathogens, 8(4), e1002588. https://doi.org/10.1371/journal.ppat.1002588

Smithburn, K. C., Hughes, T. P., Burke, A. W., & Paul, J. H. (1940). A Neurotropic Virus Isolated from the Blood of a Native of Uganda 1. The American Journal of Tropical Medicine and Hygiene, s1-20(4), 471–492. https://doi.org/10.4269/ajtmh.1940.s1-20.471

southern house mosquito - Culex quinquefasciatus Say. (n.d.). Retrieved February 17, 2025, from https://entnemdept.ufl.edu/creatures/aquatic/southern_house_mosquito.htm

Supriyono, Soviana, S., Musyaffa, M. F., Noviato, D., & Hadi, U. K. (2023). Morphological characteristic of dengue vectors Aedes aegypti and Ae. albopictus (Family: Culicidae) using advanced light and scanning electron microscope. Biodiversitas, 24(2), 894–900. https://doi.org/10.13057/biodiv/d240227

Takken, W. ., & Koenraadt, C. J. M. . (2013). Ecology of parasite-vector interactions. Wageningen Academic Publishers.

Teich, V., Arinelli, R., & Fahham, L. (2017). *Aedes aegypti* e sociedade: o impacto econômico das arboviroses no Brasil. Jornal Brasileiro de Economia Da Saúde, 9(3), 267–276. https://doi.org/10.21115/jbes.v9.n3.p267-76

Turell, M. J., O'Guinn, M. L., Dohm, D. J., & Jones, J. W. (2001). Vector Competence of North American Mosquitoes (Diptera: *Culicidae*) for West Nile Virus. Journal of Medical Entomology, 38(2), 130–134. https://doi.org/10.1603/0022-2585-38.2.130

van Slyke, G. A., Arnold, J. J., Lugo, A. J., Griesemer, S. B., Moustafa, I. M., Kramer, L. D., Cameron, C. E., & Ciota, A. T. (2015). Sequence-Specific Fidelity Alterations Associated with West Nile Virus Attenuation in Mosquitoes. PLoS Pathogens, 11(6). https://doi.org/10.1371/journal.ppat.1005009

Vasquez, M. I., Notarides, G., Meletiou, S., Patsoula, E., Kavran, M., Michaelakis, A., Bellini, R., Toumazi, T., Bouyer, J., & Petrić, D. (2023). Two invasions at once: update on the introduction of the invasive species *Aedes aegypti* and *Aedes albopictus* in Cyprus " a call for action in Europe. Parasite, 30. https://doi.org/10.1051/parasite/2023043

Vaughan, J. A., Newman, R. A., & Turell, M. J. (2022). Bird species define the relationship between West Nile viremia and infectiousness to *Culex pipiens* mosquitoes. PLoS Neglected Tropical Diseases, 16(10). https://doi.org/10.1371/journal.pntd.0010835

Verwoerd, D. W. (2015). Definition of a vector and a vector-borne disease. In Rev. Sci. Tech. Off. Int. Epiz (Vol. 34, Issue 1).

Wang, Y., Li, M., Guo, W., Deng, C., Zou, G., & Song, J. (2022). Burden of Malaria in Sao Tome and Principe, 1990–2019: Findings from the Global Burden of Disease Study 2019. International Journal of

Environmental Research and Public Health, 19(22). https://doi.org/10.3390/ijerph192214817

Weekly updates: 2024 West Nile virus transmission season. (2024, November 8). European Centre for Disease Prevention and Control. Retrieved August 30, 2024, from https://www.ecdc.europa.eu/en/west-nile-fever/surveillance-and-disease-data/disease-data-ecdc

Weeratunga, P., Rodrigo, C., Fernando, S. D., & Rajapakse, S. (2017). Control methods for Aedes albopictus and *Aedes aegypti*. Cochrane Database of Systematic Reviews, 2017(8). https://doi.org/10.1002/14651858.CD012759

Wiggins, K., Eastmond, B., & Alto, B. W. (2018). Transmission potential of Mayaro virus in Florida *Aedes aegypti* and *Aedes albopictus* mosquitoes. Medical and Veterinary Entomology, 32(4), 436–442. https://doi.org/10.1111/mve.12322

Winokur, O. C., Main, B. J., Nicholson, J., & Barker, C. M. (2020). Impact of temperature on the extrinsic incubation period of zika virus in *Aedes aegypti*. PLoS Neglected Tropical Diseases, 14(3). https://doi.org/10.1371/journal.pntd.0008047

World Bank Climate Change Knowledge Portal. (n.d.). Retrieved August 30, 2024, from https://climateknowledgeportal.worldbank.org/country/sao-tome-and-principe

World Health Organization: WHO. (2024, September 26). Vector-borne diseases. Retrieved August 30, 2024, from https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases

Wu, P., Sun, P., Nie, K., Zhu, Y., Shi, M., Xiao, C., Liu, H., Liu, Q., Zhao, T., Chen, X., Zhou, H., Wang, P., & Cheng, G. (2019). A Gut Commensal Bacterium Promotes Mosquito Permissiveness to Arboviruses. Cell Host and Microbe, 25(1), 101-112.e5. https://doi.org/10.1016/j.chom.2018.11.004

Wu, V. Y., Chen, B., Christofferson, R., Ebel, G., Fagre, A. C., Gallichotte, E. N., Sweeny, A. R., Carlson, C. J., & Ryan, S. J. (2022). A minimum data standard for vector competence experiments. Scientific Data, 9(1). https://doi.org/10.1038/s41597-022-01741-4

Yen, T.-Y., Trovoada dos Santos, M. de J., Tseng, L.-F., Chang, S.-F., Cheng, C.-F., Carvalho, A. V. de A., Shu, P.-Y., Lien, J.-C., & Tsai, K.-H. (2016). Seroprevalence of antibodies against dengue virus among pregnant women in the Democratic Republic of Sao Tome and Principe. Acta Tropica, 155, 58–62. https://doi.org/10.1016/j.actatropica.2015.12.012