Pertanika J. Trop. Agri. Sci. 46 (3): 1063 - 1081 (2023)

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Review Article

Mosquito as West Nile Virus Vector: Global Timeline of Detection, Characteristic, and Biology

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ABSTRACT

Mosquitoes are extremely important vectors that transmit zoonotic West Nile virus (WNV) globally, resulting in significant outbreaks in birds, humans, and mammals. The abundance of mosquito vectors combined with the migratory flying behaviour of wild birds across the globe has exacerbated the dynamics of WNV infection. Depth understanding of the WNV infection requires a comprehensive understanding of the character of the vector in terms of their taxonomy, morphology, biology, behaviours, preferences, and factors that promote their breeding. Most susceptible animals and humans may experience serious neurological illnesses such as encephalitis. Little is known about the susceptibility of mosquitoes to WNV

ARTICLE INFO

Article history:
Received: 01 February 2023
Accepted: 22 March 2023
Published: 30 August 2023

DOI: https://doi.org/10.47836/pjtas.46.3.18

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ISSN: 1511-3701
e-ISSN: 2231-8542

infection. This review provides insightful knowledge about the characteristics of mosquitoes that carry WNV and their susceptibility to WNV infection. The context of mosquito's involvement in WNV transmission is demonstrated through space and time from the 1950's until to date. The historical timeline of WNV transmission strength was significantly intensified via the complex interactions between vector, virus, and environment. Such knowledge will provide valuable insights into vector control intervention mitigation strategies, especially in tropical climate countries like Malaysia.

Keywords: Characteristics, mosquitoes, timeline, vectors, West Nile virus

INTRODUCTION

Approximately 3,500 mosquito species have been discovered globally, which can be categorised into 41 genera (Xia et al., 2018). Among them, some mosquitoes are associated with viruses, which have been further classified into two categories: mosquito-specific and mosquito-borne (Xia et al., 2018). Mosquito-specific viruses do not replicate in other species except mosquitoes, while mosquito-borne viruses replicate in mosquitoes and infect other animals (Bolling et al., 2015). The latter category of viruses replicates within mosquitoes and is regarded as the most significant cause of arboviral diseases, causing significant public health issues in medical and veterinary fields (Bolling et al., 2015).

West Nile virus (WNV) is an emerging, zoonotic arthropod-borne virus transmitted by mosquitoes, primarily *Culex* species, across the continent (Rizzoli et al., 2019). In 2020, a 63-year-old man was reported dead in the United States due to WNV complications (Lewis, 2020). Meanwhile, approximately 25 people were diagnosed with West Nile fever in the Seville district in Spain, while 39 and 66 cases were detected in Greece and European Union regions during the same year. About six deaths were recorded in Greece, yet the disease remained unnoticed and unresolved over time (Allen, 2020). Not only limited to temperate countries, but WNV has also been detected in tropical climate countries like Malaysia in the mosquito, humans, and animals (Ain-Najwa et al., 2020; Bowen et al., 1970; Marlina et al., 2014).

WNV is a positive-sense single-stranded RNA virion containing an icosahedral capsid with a spherical envelope grouped in the *Flaviviridae* family (Rossi et al., 2010). As the name implies, the virus was first isolated from a febrile woman in the West Nile district of Uganda in 1937 (Smithburn et al., 1940). It unexpectedly spread to the Western Hemisphere in 1999 and expanded to various regions globally (Chancey et al., 2015). WNV causes significant clinical outcomes in birds, humans, and other susceptible mammals. The infected incidental hosts are mostly dead-end hosts, resulting in various clinical manifestations ranging from predominantly asymptomatic infection to clinical syndromes such as fever and skin rashes. Severe cases involve neurological infections such as encephalitis, meningitis, and death (Rossi et al., 2010).

WNV transmission occurs in the enzootic cycle between wild birds, the reservoir host, and mosquitoes as vectors (Ahlers & Goodman, 2018). Naïve mosquitoes become infected during the blood-feeding of infected birds and subsequently transmit WNV to a wide range of susceptible hosts, including humans and animals. The renowned role of mosquitoes as vectors of WNV infection has been widely discussed. Being prevalent across the globe, including in Malaysia making, mosquitoes are an interesting subject to dig into in-depth, especially in terms of their character and effects during WNV infection. As the characteristics and susceptibility of mosquitoes in WNV infections are poorly discussed, this review will provide the information in depth.

GLOBAL TIMELINE OF WNV DETECTION IN MOSQUITO

WNV infection has been dispersed globally across Africa, Europe, North America, South America, Asia, and Australia. Various species of *Culex* spp. have been reported based on species diversity and vector competence (Figure 1). The first evidence of the detection of WNV in mosquito communities was revealed in Egypt with the discovery of *Culex univittatus* and *Culex antennatus* (Taylor et al., 1953). During the 1960s, WNV was detected in *Culex modestus* in France, isolated in humans (Hannoun et al. as cited Balenghien et al., 2008, p. 592). In Malaysia, the first study was published in 1970, at which about three *Culex* spp. (*Culex tritaeniorchynchus*, *Culex vishnui*, and *Culex pseudovishnui*) was found positive for the Kunjin virus, a subtype of WNV (Bowen et al., 1970). Later, various mosquito species was recognised, notably from *Culex* spp., as potential WNV vector in every continent up to the year 2020 in this world except Antarctica (DiMenna et al., 2006; Eastwood et al., 2011; Farajollahi et al., 2011; Hamer et al., 2009; Lu et al., 2014; Maquart et al., 2016; Martínez-de la Puente et al., 2018; Mixão et al., 2016; Orshan et al., 2008; Reisen et al., 2014).

TAXONOMY AND MOSQUITO GENUS THAT HARBOUR WNV

Mosquitoes belong to the phylum Arthropoda, class Insecta, subclass Pterygota, order Diptera, and suborder Nematocera. The Culicidae family is divided into three subfamilies, Culicinae, Anophelinae, and Toxorhynchitinae (Table 1). Culicinae (*Aedemomyia*, *Aedes*, *Armigeres*, *Coquillettidia*, *Culex*, *Culiseta*, *Ficalbia*, *Haemagogus*, *Heizmannia*, *Mansonia*, *Orthopodomyia*, *Psorophora*, *Topomyia*, *Trichoprosopon*, *Uranotaenia*, and *Verralina*) and Anophelinae (*Anopheles*) are of medical and veterinary importance as they carry deadly pathogens to animals and humans (Becker et al., 2010). The female mosquitoes from both subfamilies feed on blood to spawn progenies, contributing extensively to the spread of mosquito-borne diseases (Becker et al., 2010; Tandina et al., 2018). In contrast, members of Toxorhynchitinae feed on nectar as the source of nourishment (Taylor et al., 1953)

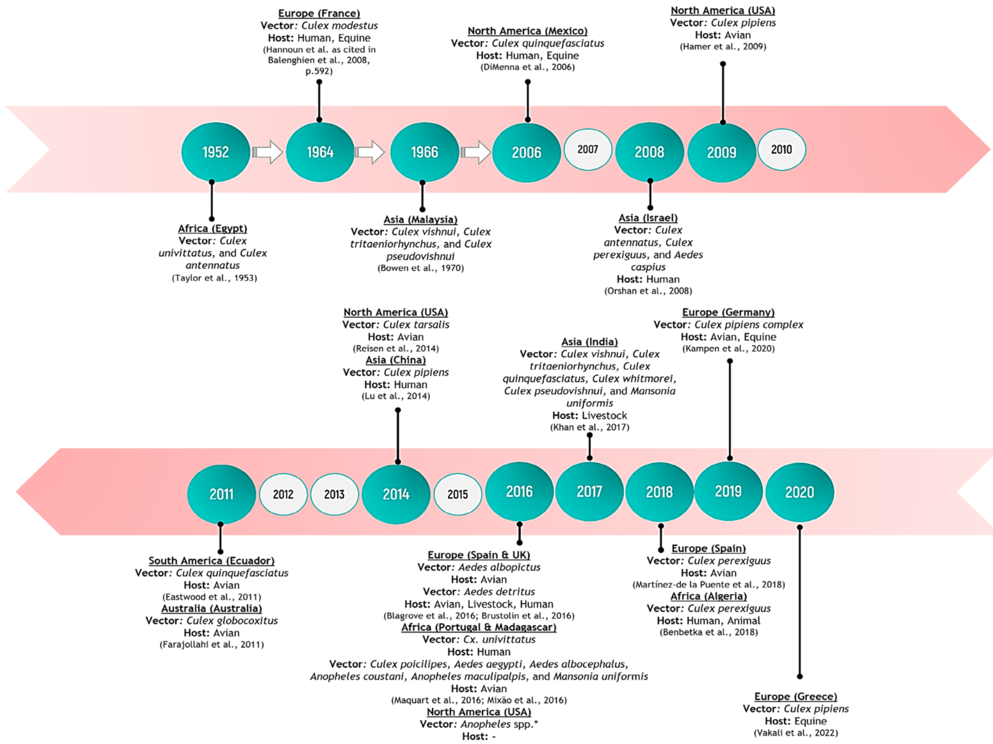

Figure 1. Global timeline of West Nile virus (WNV) vector according to species, continents, and host. This worldwide distribution was detected in different mosquito species from the year 1950's until 2020

Note. *WNV was detected in the United States from multiple countries from 1999 to 2016. *Anopheles* spp. are comprised of *Anopheles atropos*, *Anopheles barberi*, *Anopheles bradleyi*, *Anopheles franciscanus*, *Anopheles freeborni*, *Anopheles hermsi*, *Anopheles punctipennis*, *Anopheles quadrimaculatus*, and *Anopheles walkeri*

Table 1

Taxonomical classification of mosquito

Taxonomy	Classification		
Kingdom	Animalia		
Phylum	Arthropoda		
Class	Insecta		
Order	Diptera		
Suborder	Nematocera		
Family	*Culicidae*		
Subfamily	*Culicinae*	*Anophelinae*	*Toxorhynchitinae*
Genus	*Aedemomyia*, ***Aedes***, *Armigeres*, *Ayurakitia*, ***Coquillettidia***, ***Culex***, ***Culiseta***, ***Deinocerites***, *Eretmapodites*, *Ficalbia*, *Galindomyia*, *Haemagogus*,	***Anopheles***, *Bironella*, *Chagasia*	Toxorhynchites

Table 1 *(Continue)*

Taxonomy	Classification		
Genus	*Heizmannia*, *Hodgesia*, *Isostomyia*, *Johnbelkinia*, *Kimia*, *Limatus*, *Lutzia*, *Malaya*, ***Mansonia***, *Maorigoeldia*, *Mimomyia*, *Onirion*, *Opifex*, ***Orthopodomyia***, ***Psorophora***, *Runchomyia*, *Sabethes*, *Shannoniana*, *Topomyia*, *Trichoprosopon*, *Tripteroides*, ***Uranotaenia***, *Verralina*, *Wyeomyia*, *Zeugnomyia*	*Anopheles*, *Bironella*, *Chagasia*	Toxorhynchites

Note. The vectors are classified into seven taxonomic ranks. The ten genera of vectors involved in West Nile virus infection were bolded, and their distinctive features were discussed further in Table 2

and are poorly known due to the lack of medical significance (Hayes et al., 2005). Nevertheless, their larvae are used as a mosquito biological control as they prey on other mosquito larvae, gradually reducing the number of medically important mosquitoes (Service, 2012).

Mosquito species are mostly found in warm and humid climates due to their conducive environmental conditions (Paz, 2015). According to Chancey et al. (2015), although WNV has been documented throughout all continents except Antarctica, vectors of WNV among the mosquito populations are different in every continent, as presented in Figure 1. Currently, 62 mosquito species from 10 genera are involved in WNV infection, i.e., *Aedes*, *Anopheles*, *Coquillettidia*, *Culex*, *Culiseta*, *Deinocerites*, *Mansonia*, *Orthopodomyia*, *Psorophora*, and *Uranotaenia* (Centers for Disease Control and Prevention [CDC], 2016). Every genus can be differentiated through morphological features of prespiracular setae, postspiracular setae, scutellum, wing vein scales, and sterna scales that are presented on the thorax and abdomen parts (Table 2).

Laboratory experiments have demonstrated that *Aedes albopictus* and *Culex pipiens* var. *pallen* can transmit WNV (Kitaoka et al., 1950; Philip & Smadel, 1943). Additionally, Egypt reported the first isolation of WNV in 1952 from *Cx. univittatus* and *Cx. antennatus* (Taylor et al., 1953). According to Hayes et al. (2005), the abundance of mosquito vectors and the prevalence of the infection among the mosquito population is directly correlated to the severity of WNV transmission. *Culex* spp. is more frequently infected with WNV than other genera and is considered a competent vector of WNV. It was evident in a major outbreak in New York City in 1999 (Asnis et al., 2006), where *Culex* spp. was the most prominent and abundant genus compared to other genera. In the outbreak, 81% of the *Culex* spp. mosquitoes were infected with WNV with a viraemia of $10^{7.2\pm0.4}$ PFU/ml. Conversely, *Aedes* spp.

mosquitoes are classified as moderately efficient WNV vectors even though WNV was isolated from the *Aedes* spp. population during the outbreak (Turell et al., 2000). In 2002, a strain of WNV (NY99) carrying a mutation in its envelope nucleotide sequence adapted to a new *Culex* species causing a widespread epidemic involving crows, horses, and humans in the USA (Moudy et al., 2007; Snapinn et al., 2007).

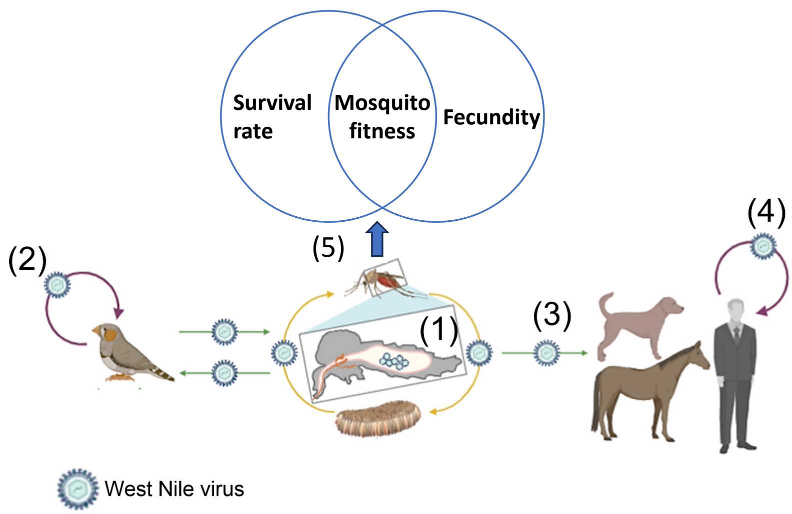

Figure 2. Overview of the West Nile virus (WNV) transmission cycle between reservoir hosts, i.e., diverse bird species and mosquitoes. Once the viraemic blood enters the body of the mosquitoes, the virus replicates in the midgut, followed by the dissemination to the salivary gland and ready to pass the virus to other hosts (1). When an infected mosquito bites the bird, the infected bird amplifies the virus and further spreads it to other birds during the roosting period (2). Concurrently, the infected mosquitoes will bite the dead-end host like humans, horses, and other mammals (3). Like in birds, the WNV transmission cycle can be circulated between humans through medical interventions such as blood transfusion, organ transplantation, and breastfeeding (4). Throughout the cycle, the mosquito population affects not only the hosts but also within the mosquito cycle via cytopathological effects in the body of mosquitoes, which are detrimental to mosquitoes with low survival rates and decreased fecundity effects (5)

Table 2

Distinctive features of mosquito genus involved in West Nile virus infection

Genus	Description	References
Aedes	Presence of postspiracular setae and dark scutum with the presence of white scales as well as broad scutellum	Becker et al. (2010); Jeffery et al. (2012)
Anopheles	Proboscis and maxillary palp have an equal length approximately with a round scutellum	Jeffery et al. (2012)
Coquillettidia	Absence on pulvilli, with yellow or purplish scutum with proboscis curved downward	Jeffery et al. (2012)

Table 2 *(Continue)*

Genus	Description	References
Culex	The absence of prespiracular and postspiracular setae on the thorax and pulvilli are present	Becker et al. (2010); Jeffery et al. (2012)
Culiseta	Presence of setae on the inferior part of mesepimeral and scale on remigium wing vein	Jeffery et al. (2012)
Deinocerites	Postspiracular setae and prespiracular setae absent Antenna longer than the proboscis	Becker et al. (2010)
Mansonia	Broad and intermixed with pale and dark on the wing vein in an asymmetrical pattern	Becker et al. (2010); Jeffery et al. (2012)
Orthopodomyia	Tarsomere leg part I is longer than the tarsomeres leg part from II-V	Jeffery et al. (2012)
Psorophora	Postspiracular setae and prespiracular setae are present. The apex of the abdomen pointed; the mesopostnotum bare	Becker et al., (2010)
Uranotaenia	Dark vertex with proboscis is swollen apically with no hair	Jeffery et al. (2012)

MORPHOLOGY OF MOSQUITOES THAT HARBOUR WNV

Mosquitoes are distinguished from other Nematocera such as Tipulidae, Bibionidae, Mycetophilidae, Sciaridae, Pschodidae, Chaoboridae, Simuliidae, Ceratopogonidae, and Chironomidae through the presence of a long-scaled proboscis feature, which is longer than the thorax and pointing forward with palps (Becker et al., 2010; Mullen & Durden, 2019). The body is divided into the head, thorax, and abdomen (Figure 3). The head consists of compound eyes, an antenna, a palp, and a proboscis (Service, 2012). The antenna and the palp are distinctive features of male and female mosquitoes and are used as sex determinants (Becker et al., 2010). Male mosquitoes have a plumose (bushy) antenna, with palp length as long as the proboscis. Female mosquitoes have a pilose (non-bushy) antenna with a shorter palp, as shown in Figure 4. A pair of pedicels is located on the head as touch and sound receptors. The proboscis is in the middle of the head in a cylindrical structure, which is responsible for injecting infected saliva and sucking the blood of hosts (Becker et al., 2010).

The thorax, which comprises three legs, each at the left and right sides of the body and a pair of wings, is placed between the head and abdomen of the mosquitoes. The vein of the wings is known as venation, which differs for some mosquito species. For instance, *Culex* spp. and *Coquillettidia* spp. wing scales are narrow and conspicuous with broad size (Becker et al., 2010). However, the wing veins are easily faded and similar in colour, causing these features to be not commonly used as key identification for species and sex determination. Eleven segments known

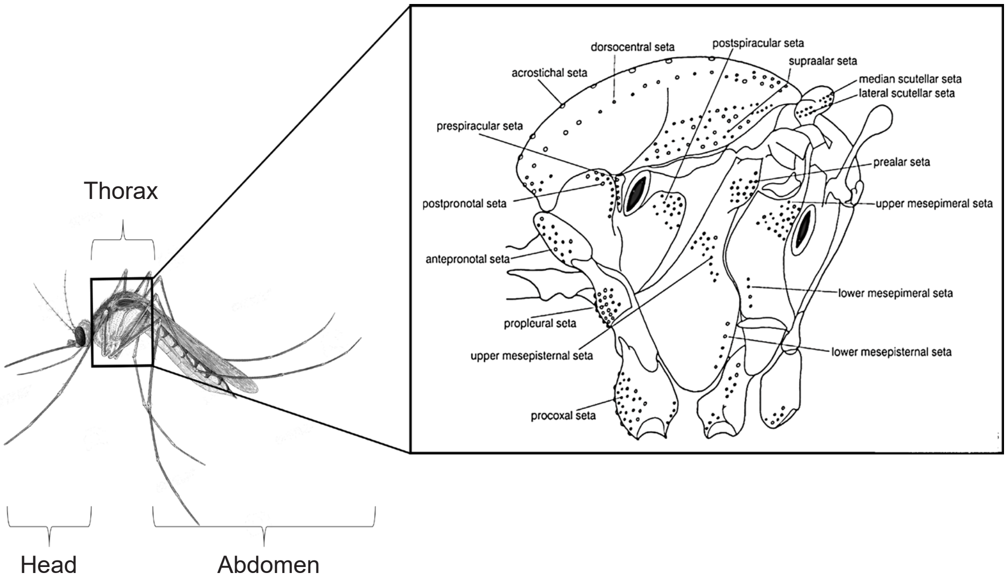

Figure 3. Morphology of mosquito. The lateral view of the thorax. The thorax is located between the head and abdomen, which comprises numerous setae in different sites to distinguish between genera and species of mosquitoes

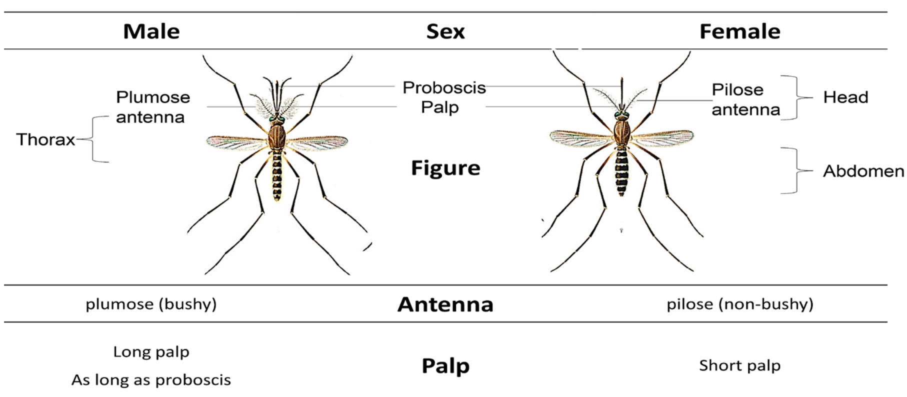

Figure 4. Male (Left) and Female (Right) *Culex* spp. mosquito based on distinctive features for sex identification, such as antenna and length of pulp (Becker et al., 2010)

as tarsals are in the abdomen and connected by an intersegmental membrane (Becker et al., 2010). The colour of the abdomen of an adult varies according to the species. For instance, *Culex* spp. mosquitoes generally appear yellowish to brown, while *Aedes* spp. normally appear black and white on the abdomen (Komp, 1923). The last tarsal comprises a pair of distinctive claspers and tiny finger-like cerci for males and females. The abdomen of an unfed female mosquito is thin and slender but expands, resembling an oval red balloon after a blood meal. The abdomen appears white when filled with eggs (Service, 2012).

BIOLOGY OF MOSQUITOES

All mosquitoes have four stages in their life cycle, i.e., the egg, larval, pupal, and adult stages, collectively known as complete metamorphosis (Becker et al., 2010). However, the development properties show great diversity between species as the mosquitoes emerge. The presence of water is necessary during the larval and pupal stages to allow them to grow (American Mosquito Control Association [AMCA], 2018). The lifespan of the mosquitoes is not more than two months on average. Typically, the life span of an adult male mosquito (≤ 10 days) is shorter than the female (Manimegalai & Sukanya, 2014). In contrast, adult female mosquitoes may live about 6 to 8 weeks in tropical regions and hibernate for 5 to 6 months in protected areas during winter (Service, 2012). These female mosquitoes come out during warm periods in the winter and lay successive batches of eggs (Lawler & Lanzaro, n.d.).

After copulation, the female mosquitoes hunt for a host to obtain blood meals for nourishment and egg maturation (Becker et al., 2010). The oviposition sites are influenced by water quality, local vegetation, light intensity, and food accessibility. The eggs are transparent initially and eventually darken to brown and black as they develop. The shape and features of the eggs are different depending on the mosquito family classification. For instance, Culicine mosquitoes lay elongated oval-shaped eggs. In contrast, Anopheline mosquitoes lay individual cigar-shaped eggs, which are laid in diverse forms, either individually or in a group adjacent to water bodies (Becker et al., 2010).

Eggs of most mosquito genera have a lengthy survival period but may easily die due to desiccation, except for *Aedes* spp., which can withstand a longer desiccation period (Becker et al., 2010). Greater chitin content and melanisation degree enhanced the egg resistance desiccation (ERD) level. Hence, *Aedes*' egg showed higher contents of chitin and melanin than other mosquito genera, contributing to its increased resilience towards desiccation under inhospitable environments (Farnesi et al., 2017). The species can withstand desiccation for up to 8 months (Kweka et al., 2018). Once the eggs are hatched, the larval stage develops and undergoes four instars before developing into the pupal stage. The larva inhabits aquatic conditions and continues to evolve in water bodies (Bashar et al., 2016). The size of the larva increases with every instar stage when they moult the outer skin. The level of each instar is determined by the head capsule size located anterior to the larvae. The tail, known as the siphon, is located on the posterior site and functions as a breathing apparatus via spiracles (AMCA, 2018). The breathing process occurs by having the larva hanging downward while exposing the siphon to the air. In water bodies, mosquitoes feed on algae, protozoa, and microorganisms as their source of nutrients (Becker et al., 2010; Diaz-Nieto et al., 2016). The development of the mosquito larval stage depends on environmental temperatures (Becker et al., 2010). For instance, the floodwater mosquitoes, such as *Aedes vexans*, successfully develop at 30°C, while snow-melt mosquitoes like *Ochlerotatus cantans* prefer lower temperatures (~10°C) and cannot develop at temperatures over 25°C. In contrast, the larvae of *Cx. pipiens* thrive between 10 and 30°C (Becker et al., 2010).

The pupal stage continues after the fourth instar stage in the aquatic habitat. The pupa does not feed and can withstand a short period of desiccation. Tissue destruction of larval organs occurs prior to the development of the adult body within the pupal skin (Becker et al., 2010).

Once the adult emerges, the haemolymph pressure is raised for the legs and wings to stretch. The emerging males come out 1 to 2 days before the female completes sexual maturity concurrently with the emerging female. The mosquitoes take a short break on the water's surface before flying off to allow the body and wings to dry. After 24 to 48 hr of emergence, female and male adult mosquitoes feed on nectar before mating (Becker et al., 2010).

TRANSMISSION OF WNV

Horizontal transmission between the avian reservoirs, mostly from the family of passerine and charadriiform and the mosquitoes, primarily the *Culex* and *Aedes* genera, had contributed to the large outbreak of WNV in the USA (Komar et al., 2003). However, this transmission mode only partially explained the expansion of WNV infections. Other modes of transmission, such as vertical transmission, are important for maintaining WNV among mosquito populations, as shown in Figure 2 (J. F. Anderson & Main, 2006).

Vertical transmission depends on vector competence, which is affected by extrinsic and intrinsic factors. The extrinsic factors comprise virus dosage (Kramer et al., 1983) and extrinsic incubation temperature (Dohm et al., 2002). The extrinsic incubation period, which begins when the mosquito ingests the virus through an infected blood meal and ends when the host is infected (S. L. Anderson et al., 2010), is also crucial. Meanwhile, the intrinsic factors affecting vertical transmission are comprised of virus strains (Moudy et al., 2007), mosquito populations (Richards et al., 2007), and mosquito species (Goddard et al., 2003).

Consequently, mosquitoes are greatly affected by infection and resistance to WNV infection (Ciota et al., 2011). Studies reported mosquito-specific species influenced these conditions as measured parameters for determining the potential vector of WNV (Ciota et al., 2011). For instance, no significant differences in the survival and fecundity rates are observed, measured by the number of egg production and hatched eggs in WNV-infected female *Cx. pipiens* and unexposed female *Cx. pipiens* (Styer et al., 2007). Conversely, WNV-infected female *Culex tarsalis* experienced reduced fertility and increased blood-feeding rates (Ciota et al., 2011).

Nevertheless, WNV could replicate higher viral loads in *Cx. tarsalis* compared to *Cx. pipiens*, with up to $10^{7.7}$ PFU/ml, recovered under diverse experimental conditions (Dohm et al., 2002; Styer et al., 2007). Distinctive viral loads were detected in four different body segments of the mosquito, including the head, thorax, abdomen, and leg segments, suggesting widespread dissemination to other body parts. The study estimated that the thorax, where the salivary gland is located, contained the highest viral load of WNV, ranging from $10^{5.5}$ to 10^{7} PFU/ml, followed by the head ($10^{4.8}$–10^{6} PFU/ml), abdomen ($10^{4.6}$–$10^{6.1}$ PFU/ml), and leg segment ($10^{3.5}$–10^{5} PFU/ml) (Styer et al., 2007).

The adaptation of the mosquito fitness towards WNV infection is shielded by the immune response of mosquito immunity against WNV, primarily on the salivary gland and midgut part (Ahlers & Goodman, 2018; Sim et al., 2014). The blood-feeding process begins with the insertion of the proboscis, a long thin needle-like structure found in the mouthpart of the mosquito. The proboscis comprises six stylets, including a hypopharynx, labrum, mandibles, and maxillae (Kong & Wu, 2010). It vertically pierces the host's skin (dermis) and injects saliva. The saliva contains heparin, acting as the anticoagulant (Ha et al., 2014), and sialokinin to increase endothelial permeability, which leads to vasodilation (Zeidner et al., 1999) and provides the anaesthetic effect (Ribeiro & Francischetti, 2003). During this process, the susceptible vector is infected with WNV from ingesting the viraemic blood meal of the amplifying host. A viral load at approximately 4.4 logs PFU WNV/ml is enough for the mosquitoes to establish WNV infection (Richards et al., 2007). Replication of the virus takes place in the midgut, followed by transfer to the salivary gland and dissemination throughout the mosquito body (Cheng et al., 2016). However, the midgut and salivary glands can act as barriers that impede the dissemination of the virus throughout the mosquito's body. As a result, not all mosquitoes serve as the vector of WNV (Vogels et al., 2017). The limitation of virus replication is species-specific, resulting in the distinct vector species being susceptible to the respective mosquito-borne viruses (Wanasen et al., 2004). The mosquito immune system and RNAi pathway in the innate immune response of the mosquito are protective, warding off death from WNV infection (Ahlers & Goodman, 2018). However, the maximum capacity for the different mosquito species to replicate WNV is unknown.

Unlike the high viral load in mosquitoes, dead-end hosts such as mice could only recover low amounts of WNV viral load (10^{2} PFU/ml of blood), impeding WNV transmission (Styer et al., 2007). Thus, other mammals, including humans, are specified as dead-end hosts for WNV, likely the endpoint in the WNV transmission cycle (Colpitts et al., 2012). Immunocompromised dead-end hosts could potentially develop higher levels of viraemia. For example, dogs treated with methylprednisolone (glucocorticoid) showed higher mean WNV titres than the control group, with up to 6.6×10^{8} PFU/mosquito values. Similarly, immunosuppressed hamsters treated with cyclophosphamide, one of the chemical compounds used in chemotherapy, had increased WNV viraemic load, which continued to elevate for eight days post-infection of WNV, resulting in serious illness or death (Mateo et al., 2006). The exposure to the compound caused a decrease in the total leukocyte count, signifying that the animals were immunocompromised and developing clinical signs affecting several vital organs, i.e., the lungs, heart, kidney, and parts of the brain, involving the hippocampus, cerebral cortex, and cerebellum sections. Besides, high WNV titres (10^8 PFU/ml) were sustained for 8 days in the WNV-infected immunosuppressed hamster before death compared to the control hamster (Mateo et al., 2006).

BREEDING PREFERENCE OF MOSQUITOES THAT HARBOUR WNV

The ubiquity of adult mosquitoes in a habitat is dependent on diverse factors, including the host population and biotic and abiotic factors (Bashar et al., 2016). The lack of a hygienic environment contributes to various conducive breeding sites for mosquito species such as *Culex quinquefasciatus* and *Aedes aegypti* (Bashar et al., 2016). *Culex* mosquitoes are primarily abundant in peri-urban and rural areas due to continuous water sources from irrigation systems (Boyer et al., 2014). Water depth affects the suitability of the breeding habitat of different mosquito species. *Aedes aegypti* and *Ae. albopictus* are found at low water depth, whereas *Cx. quinquefasciatus* and *Culex tritaeniorhynchus* prefer high water depth for oviposition (Bashar et al., 2016). Additionally, Rueda et al. (2008) reported that freshwater and brackish water are preferable habitats for mosquitoes to thrive in, but they do not breed in marine habitats with high-salt concentrations. A study by Tandina et al. (2018) reported that, *Ae. aegypti* mosquitoes favour clean water with average organic content, which usually collects in tree holes and man-made containers. The abundance of artificial and natural containers has increased the habitats suitable for *Aedes* mosquitoes to grow (Tandina et al., 2018). Urbanisation and the abundance of vegetation contributed to increased habitats for *Aedes* spp. mosquitoes primarily, *Ae. aegypti* (Ebi & Nealon, 2016). It shows that every mosquito species has a distinct feeding habit. The *Culex* species are more active from dusk to dawn and are known as night-biting mosquitoes (Becker et al., 2010). According to Rohani et al. (2008), the *Culex* species favours polluted aquatic environments such as cesspools.

FACTORS THAT PROMOTE THE BREEDING OF MOSQUITOES AND ENHANCE WNV TRANSMISSION

Aquatic Site

According to Maquart et al. (2016), lakeshores with emergent vegetation promote the breeding of mosquitoes and present a higher number of positive WNV mosquito pools. However, there are WNV infections at sites far from lakeshores, even though the number of mosquito traps was lowered. Additionally, migratory birds are preferred to stay aloft in small lakes and swamp areas for long or short rest where there are higher numbers of *Cx. pipiens* mosquito populations, the most WNV-competent vector (Gomes et al., 2013).

Migration of Wild Birds

WNV intensification and transmission by mosquito fully depends on introducing an immunologically naïve avian population (Reisen et al., 2014). Dispersion of birds into new geographic attributes to the prompt widespread of WNV transmission in North America in 1999 (Rappole, 2000). Various migratory and resident birds choose wetland habitats as resting and breeding sites, permitting the virus to be established through the migration route, which eventually allows rapid augmentation of WNV via enzootic transmission (Valiakos et al., 2014).

Ornithophilic Properties

The ornithophilic properties of some species of mosquitoes, such as *Cx. univittatus* that also feed on humans for their blood meal increases the potential threat of the species as WNV vectors (Mixão et al. 2016). Further, the blood preferences of *Cx. pipiens* are avian hosts, mainly from the Passeriformes family: Blue Jay, Common Grackle, House Finch, American Crow, House Sparrow, Cardinal, and American Robin. These bird species attribute to the reservoir host of the WNV transmission and viral expansion of WNV (Komar et al., 2003).

Climatic Condition

In terms of climate, the ambient temperature increases the transmission of WNV and vector abundance (Deichmeister & Telang, 2011). WNV was discovered throughout tropical and temperate regions of the world, which are conducive climates for *Cx. pipiens* and *Cx. quinquefasciatus* to breed and survive (Farajollahi et al., 2011). Environmental factors, mainly temperature and rainfall parameters are crucial in breeding mosquitoes and WNV transmission (Paz, 2015). Flooding and heavy rainfall are ideal environments for ardeid birds, which subsequently lead to the proliferation of the *Culex annulirostris* mosquito (van den Hurk et al., 2014). Further, higher temperatures increased the infectivity on the rate of virus replication (Kilpatrick et al., 2008).

Anthropogenic Activities

Anthropogenic activities involve the transformation in nature, which results from human activity such as deforestation, urbanisation, and global warming. Xia et al. (2018) revealed that the global warming and climate change phenomenon is prone to the indirect transfer of infected vectors, causing a higher number of mosquito viruses in China. Importantly in terms of vector ecology, *Culex* spp. mosquitoes prone to well adapted to the man-made habitat and rise in vector abundance, ultimately increasing the avian seroprevalence in urban sites (Epstein, 2001). Deforestation disrupts the natural habitat of vectors and influences the transmission of vector-borne diseases (Burkett-Cadena & Vittor, 2018). Besides, the emergence of puddles in deforested sites creates a favourable location for the development of larvae (Vora, 2008).

CONCLUSION

Successful WNV transmission from the amplifier host to the susceptible host has been largely contributed by the strong and impactful role of mosquitoes as vectors. By recognising the taxonomic and morphological characteristics of *Culex* spp. as the principal vector of WNV, its ornithophilic properties and factors that enhance their breeding provide a better understanding of WNV transmission and infection dynamics globally, including in Malaysia.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

ACKNOWLEDGEMENTS

This work is supported by the Ministry of Higher Education Grant, Malaysia — France Bilateral Research Collaboration 2021 (MATCH 2021) with the grant number KPT MATCH/2021/5540495 under Project *In vitro* Differential Neuropathogenicity of Nonstructural Proteins (NSPs) of West Nile virus isolated from Southeast Asia and Europe and Universiti Putra Malaysia Grantmanship — Geran Putra Berimpak (UPM.RMC.800/2/2/4-GPB).

REFERENCES

Ahlers, L. R. H., & Goodman, A. G. (2018). The immune responses of the animal hosts of West Nile virus: A comparison of insects, birds, and mammals. *Frontiers in Cellular and Infection and Microbiology*, *8*, 96. https://doi.org/10.3389/fcimb.2018.00096

Ain-Najwa, M. Y., Yasmin, A. R., Omar, A. R., Arshad, S. S., Abu, J., Mohammed, H. O., Kumar, K., Loong, S. K., Rovie-Ryan, J. J., & Mohd-Kharip-Shah, A.-K. (2020). Evidence of West Nile virus infection in migratory and resident wild birds in west coast of peninsular Malaysia. *One Health*, *10*, 100134. https://doi.org/10.1016/j.onehlt.2020.100134

Allen, N. (2020). *West Nile virus outbreak kills two in southern Spain*. Reuters. https://www.reuters.com/article/us-health-nile-fever-spain/west-nile-virus-outbreak-kills-two-in-southern-spain-idINKBN25H1KY

American Mosquito Control Association. (2018). *Mosquito biology*. AMCA. https://www.mosquito.org/page/mosquitoinfo

Anderson, J. F., & Main, A. J. (2006). Importance of vertical and horizontal transmission of West Nile virus by *Culex pipiens* in the Northeastern United States. *The Journal of Infectious Diseases*, *194*(11), 1577-1599. https://doi.org/10.1086/508754

Anderson, S. L., Richards, S. L., & Smartt, C. T. (2010). A simple method for determining arbovirus transmission in mosquitoes. *Journal of the American Mosquito Control Association*, *26*(1), 108-111. https://doi.org/10.2987/09-5935.1

Asnis, D. S., Conetta, R., Waldman, G., & Teixeira, A. A. (2006). The West Nile virus encephalitis outbreak in the United States (1999-2000): From flushing, New York, to beyond its borders. *Annals of the New York Academy of Sciences*, *951*(1), 161-171. https://doi.org/10.1111/j.1749-6632.2001.tb02694.x

Balenghien, T., Vazeille, M., Grandadam, M., Schaffner, F., Zeller, H., Reiter, P., Sabatier, P., Fouque, F., & Bicout, D. J. (2008). Vector competence of some French *Culex* and *Aedes* mosquitoes for West Nile virus. *Vector-Borne and Zoonotic Diseases*, *8*(5), 589-596. https://doi.org/10.1089/vbz.2007.0266

Bashar, K., Rahman, M. S., Nodi, I. J., & Howlader, A. J. (2016). Species composition and habitat characterization of mosquito (Diptera: Culicidae) larvae. *Pathogen and Global Health*, *110*(2), 48-61. https://doi.org/10.1080/20477724.2016.1179862

Becker, N., Petric, D., Zgomba, M., Boase, C., Madon, M., Dahl, C., & Kaiser, A. (2010). *Mosquito and their control*. Springer. https://doi.org/10.1007/978-3-540-92874-4

Benbetka, S., Hachid, A., Benallal, K. E., Benbetka, C., Khaldi, A., Bitam, I., & Harrat, Z. (2018). First field evidence infection of *Culex perexiguus* by West Nile virus in Sahara Oasis of Algeria. *Journal of Vector Borne Diseases*, *55*(4), 305-309. https://doi.org/10.4103/0972-9062.256566

Blagrove, M. S., Sherlock, K., Chapman, G. E., Impoinvil, D. E., McCall, P. J., Medlock, J. M., Lycett, G., Solomon, T., & Baylis, M. (2016). Evaluation of the vector competence of a native UK mosquito *Ochlerotatus detritus* (*Aedes detritus*) for dengue, chikungunya and West Nile viruses. *Parasites and Vectors*, *9*, 452. https://doi.org/10.1186/s13071-016-1739-3

Bolling, B. G., Weaver, R. B., Tesh, R. B., & Vasilakis, N. (2015). Insect-specific virus discovery: Significance for the arbovirus community. *Viruses*, *7*(9), 4911-4928. https://doi.org/10.3390/v7092851

Bowen, E. T. W., Simpson, D. I. H., Platt, G. S., Way, H. J., Gordon-Smith, C. E., Ching, C. Y., & Casals, J. (1970). Arbovirus infections in Sarawak: The isolation of Kunjin virus from mosquitoes of the *Culex pseudovishnui* group. *Annals of Tropical Medicine and Parasitology*, *64*(3), 263-268.

Boyer, S., Luciano, T. M., Randriamaherijaona, S., Andrianaivolambo, L., & Cardinale, E. (2014). Mosquitoes sampling strategy for studying West Nile virus vectors in Madagascar: Abundance, distribution and methods of catching in high risk areas. *Archives de I'Institut Pasteur De Madagascar*, *71*, 1–4.

Brustolin, M., Talavera, S., Santamaría, C., Rivas, R., Pujol, N., Aranda, C., Marquès, E., Valle, M., Verdún, M., Pagès, N., & Busquets, N. (2016). *Culex pipiens* and *Stegomyia albopicta* (= *Aedes albopictus*) populations as vectors for lineage 1 and 2 West Nile virus in Europe. *Medical and Veterinary Entomology*, *30*(2), 166-173. https://doi.org/10.1111/mve.12164

Burkett-Cadena, N. D., & Vittor, A. Y. (2018). Deforestation and vector-borne disease: Forest conversion favors important mosquito vectors of human pathogens. *Basic and Applied Ecology*, *26*, 101-110. https://doi.org/10.1016/j.baae.2017.09.012

Centers for Disease Control and Prevention. (2016). *Mosquito species in which West Nile virus has been detected, United States, 1999-2016*. CDC. https://www.cdc.gov/westnile/resources/pdfs/MosquitoSpecies1999-2016.pdf

Chancey, C., Grinev, A., Volkova, E., & Rios, M. (2015). The global ecology and epidemiology of West Nile virus. *BioMed Research International*, *2015*, 376230. https://doi.org/10.1155/2015/376230

Cheng, G., Liu, Y., Wang, P., & Xiao, X. (2016) Mosquito defense strategies against viral infection. *Trends in Parasitology*, *32*(3), 177-186. https://doi.org/10.1016/j.pt.2015.09.009

Ciota, A. T., Styer, L. M., Meola, M. A., & Kramer, L. D. (2011) The costs of infection and resistance as determinants of West Nile virus susceptibility in *Culex* mosquitoes. *BMC Ecology*, *11*, 23. https://doi.org/10.1186/1472-6785-11-23

Colpitts, T. M., Conway, M. J., Montgomery, J. J., & 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

Deichmeister, J. M., & Telang, A. (2011). Abundance of West Nile virus mosquito vectors in relation to climate and landscape variables. *Journal of Vector Ecology*, *36*(1), 75-85. https://doi.org/10.1111/j.1948-7134.2011.00143.x

Diaz-Nieto, L. M., D'Aleessio, C., Perotti, M. A., & Berón, C. M. (2016). *Culex pipiens* development is greatly influenced by native bacteria and exogenous yeast. *PLOS One*, *11*(4), e0153133. https://doi.org/10.1371/journal.pone.0153133

DiMenna, M. A., Bueno, R., Parmenter, R. R., Norris, D. E., Sheyka, J. M., Molina, J. L., LaBeau, E. M., Hatton, E. S., & Glass, G. E. (2006). Emergence of West Nile virus in mosquito (Diptera: Culicidae) communities of the New Mexico Rio Grande Valley. *Journal of Medical Entomology*, *43*(3), 594-599. https://doi.org/10.1603/0022-2585(2006)43[594:EOWNVI]2.0.CO;2

Dohm, D. J., Sardelis, M. R., & Turell, M. J. (2002). Experimental vertical transmission of West Nile virus by *Culex pipiens* (Diptera: Culicidae). *Journal of Medical Entomology*, *39*(4),640-644. https://doi.org/10.1603/0022-2585-39.4.640

Eastwood, G., Kramer, L. D., Goodman, S. J., & Cunningham, A. A. (2011). West Nile virus vector competency of *Culex quinquefasciatus* mosquitoes in the Galápagos Islands. The *American Journal of Tropical Medicine and Hygiene*, *85*(3), 426-433. https://doi.org/10.4269/ajtmh.2011.10-0739

Ebi, K. L., & Nealon, J. (2016). Dengue in a changing climate. *Environmental Research*, *151*, 115-123. https://doi.org/10.1016/j.envres.2016.07.026

Epstein P. R. (2001). West Nile virus and the climate. *Journal of Urban Health*: *Bulletin of the New York Academy of Medicine*, *78*(2), 367–371. https://doi.org/10.1093/jurban/78.2.367

Farajollahi, A., Fonseca, D. M., Kramer, L. D., & Kilpatrick, A. M. (2011). Bird biting mosquito and human disease: A review of the role of *Culex pipiens* complex mosquitoes in epidemiology. *Infection Genetics and Evolutions*, *78*(2), 1577-1585. https://doi.org/10.1016/j.meegid.2011.08.013

Farnesi, L. C., Vargas, H. C. M., Valle, D., & Rezende, G. L., (2017). Darker eggs of mosquitoes resist more to dry conditions: Melanin enhances serosal cuticle contribution in egg resistance to desiccation in *Aedes*, *Anopheles* and *Culex* vectors. *PLOS Neglected Tropical Disease*, *11*(10), e0006063. https://doi.org/10.1371/journal.pntd.0006063

Goddard, L. B., Roth, A. E., Reisen, W. K., & Scott, T. W. (2003). Vertical transmission of West Nile virus by three California *Culex* (Diptera: Culicidae) species. *Journal of Medical Entomology*, *40*(6), 743–746. https://doi.org/10.1603/0022-2585-40.6.743

Gomes, B., Sousa, C. A., Vicente, J. L., Pinho, L., Calderón, I., Arez, E., Almeida, A. P. G., Donnelly, M. J., & Pinto, J. (2013). Feeding patterns of molestus and pipiens forms of *Culex pipiens* (Diptera: Culicidae) in a region of high hybridization. *Parasites and Vectors*, *6*, 93. https://doi.org/10.1186/1756-3305-6-93

Ha, Y.-R., Oh, S.-R., Seo, E.-S., Kim, B.-H., Lee, D.-K., & Lee S.-J. (2014). Detection of heparin in the salivary gland and midgut of *Aedes togoi*. *The Korean Journal of Parasitology*, *52*(2), 183-188. https://doi.org/10.3347/kjp.2014.52.2.183

Hamer, G. L., Kitron, U. D., Goldberg, T. L., Brawn, J. D., Loss, S. R., Ruiz, M. O., Hayes D. B., & Walker, E. D. (2009). Host selection by *Culex pipiens* mosquitoes and West Nile virus amplification. *The American Journal of Tropical Medicine and Hygiene*, *8*0(2), 268-278. https://doi.org/10.4269/ajtmh.2009.80.268

Hayes, E. B., Komar, N., Nasci, R. S., Montgomery, S. P., O'Leary, D. R., & Campbell, G. L. (2005). Epidemiology and transmission dynamics of West Nile virus disease. *Emerging Infectious Diseases*, *11*(8), 1167-1173. https://doi.org/10.3201/eid1108.050289a

Jeffery, J., Rohela, M., Muslimin, M., Abdul Aziz, S. M. N., Jamaiah, I., Kumar, S., Tan, T. C., Lim, Y. A. L., Nissapatorn, V., & Abdul-Azizi, N. M. (2012). *Illustrated keys: Some mosquitoes of Peninsula Malaysia, Malaysia*. Universiti Malaya Press.

Kampen, H., Holicki, C. M., Ziegler, U., Groschup, M. H., Tews, B. A., & Werner, D. (2020). West Nile virus mosquito vectors (Diptera: Culicidae) in Germany. *Viruses*, *12*(5), 493. https://doi.org/10.3390/v12050493

Khan, S. A., Chowdhury, P., Choudhury, P., & Dutta, P. (2017). Detection of West Nile virus in six mosquito species in synchrony with seroconversion among sentinel chickens in India. *Parasites and Vectors*, *10*(1), 13. https://doi.org/10.1186/s13071-016-1948-9

Kilpatrick, A. M., Meola, M. A., Moudy, R. M., & Kramer, L. D. (2008). Temperature, viral genetics and the transmission of West Nile virus by *Culex pipiens* mosquitoes. *PLOS Pathogen*, *4*(6), e1000092. https://doi.org/10.1371/journal.ppat.1000092

Kitaoka, M. (1950). Experimental transmission of the West Nile virus by the mosquito. *The Japanese Medical Journal*, *3*(2), 77-81. https://doi.org/10.7883/yoken1948.3.77

Komar, N., Langevin, S., Hinten, S., Nemeth, N., Edwards, E., Hettler, D., Davis, B., Bowen, R., & Bunning, M. (2003). Experimental infection of North American birds with the New York 1999 strain of West Nile virus. *Emerging Infectious Diseases*, *9*(3), 311-322. https://doi.org/10.3201/eid0903.020628

Komp, W. H. W. (1923). Guide to mosquito identification for field workers engaged in Malaria control in the United States. *Public Health Reports*, *38*(20), 1061-1080. https://doi.org/10.2307/4576745

Kong, X. Q., & Wu, C. W. (2010). Mosquito proboscis: An elegant biomicroelectromechanical system. *Physical Review E: Statistical, Nonlinear, and Soft Matter Physics*, *82*(1), 011910. https://doi.org/10.1103/PhysRevE.82.011910

Kramer, L. D., Hardy, J. L., & Presser, S. B. (1983). Effect of temperature of extrinsic incubation on the vector competence of *Culex tarsalis* for western equine encephalomyelitis virus. *The American Journal of Tropical Medicine and Hygiene*, *32*(5), 1130-1139. https://doi.org/10.4269/ajtmh.1983.32.1130

Kweka, E. J., Baraka, V., Mathias, L., Mwang'onde, B., Baraka, G., Lucile, L., & Mahande, A. M., (2018). Ecology of *Aedes* mosquitoes, the major vectors of arboviruses in human population. In J. A. Falcón-Lezama, M. Betancourt-Cravioto, & R. Tapia-Conyer (Eds.), *Dengue fever — A resilient threat in the face of innovation* (pp. 39-55). IntechOpen. https://doi.org/10.5772/intechopen.81439

Lawler, S. P., & Lanzaro, G. C. (n.d.). *Managing mosquitoes on the farm*. https://s3.wp.wsu.edu/uploads/sites/2061/2014/01/MosquitosOnTheFarm.pdf

Lewis, I. (2020, October 9). Clark Middleton: Twin Peaks and The Blacklist actor dies aged 63 of West Nile virus. *Independent*. https://www.independent.co.uk/arts-entertainment/tv/news/clark-middleton-death-blacklist-twin-peaks-west-nile-virus-b828450.html

Lu, Z., Fu, S.-H., Cao, L., Tang, C.-J., Zhang, S., Li, Z.-X., Tusong, M., Yao, X.-H., Zhang, H.-L., Wang, P.-Y., Wumaier, M., Yuan, X.-Y., Li, M.-H., Zhu, C.-Z., Fu, L.-P., & Liang, G.-L. (2014). Human infection with West Nile virus, Xinjiang, China, 2011. *Emerging Infectious Diseases*, *20*(8), 1421-1423. https://doi.org/10.3201%2Feid2008.131433

Manimegalai, K., & Sukanya, S., (2014). Biology of the filarial vector, *Culex quinquefasciatus* (Diptera: Culicidae). *International Journal of Current Microbiology and Applied Sciences*, *3*(4), 718-724.

Maquart, M., Boyer, S., Rakotoharinome, V. M., Ravaomanana, J., Tantely, M. L., Heraud, J., & Cardinale, E. (2016). High prevalence of West Nile virus in domestic birds and detection in 2 new mosquito species in Madagascar. *PLOS One*, *11*(1), e0147589. https://doi.org/10.1371/journal.pone.0147589

Marlina, S., Radzi, S. F., Lani, R., Sieng, K. C., Rahim, N. F., Hassan, H., Li-Yen, C., AbuBakar, S., & Zandi, K. (2014). Seroprevalence screening for the West Nile virus in Malaysia's Orang Asli population. *Parasites and Vectors*, *7*, 597. https://doi.org/10.1186/s13071-014-0597-0

Martínez-de la Puente, J., Ferraguti, M., Ruiz, S., Roiz, D., Llorente, F., Pérez-Ramírez, E., Jiménez-Clavero, M. A., Soriguer, R., & Figuerola, J. (2018). Mosquito community influences West Nile virus seroprevalence in wild birds: Implications for the risk of spillover into human populations. *Scientific Reports*, *8*, 2599. https://doi.org/10.1038/s41598-018-20825-z

Mateo, R, Xiao, S.-Y., Guzman, H., Lei, H., da Rosa, A. P. T., & Tesh, R. B. (2006). Effects of immunosuppression on West Nile virus infection in hamsters. *The American Journal of Tropical Medicine and Hygiene*, *75*(2), 356-362. https://doi.org/10.4269/ajtmh.2006.75.356

Mixão, V., Barriga, D. B., Parreira, R., Novo, M. T., Sousa, C. A., Frontera, E., Venter, M., Braack, L., & Almeida, A. P. G. (2016). Comparative morphological and molecular analysis confirms the presence of the West Nile virus mosquito vector *Culex univittatus*, in the Siberian peninsula. *Parasites and Vectors*, *9*, 601. https://doi.org/10.1186/s13071-016-1877-7

Moudy, R. M., Meola M. A., Morin, L. L., Ebel, G. D., & Kramer, L. D. (2007). A newly emergent genotype of West Nile virus is transmitted earlier and more efficiently by *Culex* mosquitoes. *American Journal of Tropical Medicine and Hygiene*, *77*(2), 365-370.

Mullen, G. R., & Durden, L. A. (Eds.) (2019). *Medical and veterinary entomology* (3rd ed.). Academic Press. https://doi.org/10.1016/C2017-0-00210-0

Orshan, L., Bin, H., Schnur, H., Kaufman, A., Valinsky, A., Shulman, L., Weiss, L., Mendelson, E., & Pener, H. (2008). Mosquito vectors of West Nile fever in Israel. *Journal of Medical Entomology*, *45*(5), 939-947. https://doi.org/10.1603/0022-2585(2008)45[939:mvownf]2.0.co;2

Paz, S. (2015). Climate change impacts on West Nile virus transmission in a global context. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1665), 20130561. https://doi.org/10.1098/rstb.2013.0561

Philip, C. B., & Smadel, J. E. (1943). Transmission of West Nile virus by infected *Aedes albopictus*. *Experimental Biology and Medicine*, *53*(1), 49-50. https://doi.org/10.3181/00379727-53-14180

Rappole, J. H., Derrickson, S. R., & Hubálek, Z. (2000). Migratory birds and spread of West Nile virus in the Western Hemisphere. *Emerging Infectious Diseases*, *6*(4), 319-328. https://doi.org/10.3201%2Feid0604.000401

Reisen, W. K., Fang, Y., & Martinez, V. M. (2014). Effects of temperature on the transmission of West Nile virus by *Culex tarsalis* (Diptera: Culicidae). *Journal of Medical Entomology*, *43*(2), 309-317. https://doi.org/10.1093/jmedent/43.2.309

Ribeiro, J. M. C., & Francischetti, I. M. B. (2003). Role of arthropod saliva in blood feeding: Sialome and post-sialome perspectives. *Annual Review of Entomology*, *48*, 73-88. https://doi.org/10.1146/annurev.ento.48.060402.102812

Richards, S. L., Mores, C. N., Lord, C. C., & Tabachnick, W. J. (2007). Impact of extrinsic incubation temperature and virus exposure on vector competence of *Culex pipiens quinquefasciatus* Say (Diptera: Culicidae) for West Nile virus. *Vector Borne for Zoonotic Diseases*, *7*(4), 629-636. https://doi.org/10.1089/vbz.2007.0101

Rizzoli, A., Tagliapietra, V., Cagnacci, F., Marini, G., Arnoldi, D., Rosso, F., & Rosà, R. (2019). Parasites and wildlife in a changing world: The vector-host-pathogen interaction as a learning case. *International Journal for Parasitology: Parasites and Wildlife*, *9*, 394-401. https://doi.org/10.1016/j.ijppaw.2019.05.011

Rohani, A., Chan, S. T., Abdullah, A. G., Tanrag, H., & Lee, H. L. (2008). Species composition of mosquito fauna in Ranau, Sabah, Malaysia. *Tropical Biomedicine*, *25*(3), 232-236.

Rossi, S. L., Ross, T. M., & Evans, J. D. (2010). West Nile Virus. *Clinics in Laboratory Medicine*, *30*(1), 47-65. https://doi.org/10.1016/j.cll.2009.10.006

Rueda, L. M. (2008). Global diversity of mosquitoes (Insecta: Diptera: Culicidaee) in freshwater. *Hydrobiologia*, *595*, 477-487. https://doi.org/10.1007/s10750-007-9037-x

Service, M. (2012). Introduction to mosquitoes (Culicidae). In *Medical entomology for students* (pp. 1-32). Cambridge University Press. https://doi.org/10.1017/CBO9780511811012.005

Sim, S., Jupatanakul, N., & Dimopoulos, G. (2014). Mosquito immunity against arboviruses. *Viruses*, *6*(11), 4479–4504. https://doi.org/10.3390/v6114479

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. *The American Journal of Tropical and Medicine and Hygiene*, *20*, 471-472.

Snapinn, K. W., Holmes, E. C., Young, D. S., Bernard, K. A., Kramer, L. D., & Ebel, G. D. (2007). Declining growth rate of West Nile virus in North America. *Journal of Virology*, *81*(5), 2531-2534. https://doi.org/10.1128/JVI.02169-06

Styer, L. M., Meola, M. A., & Kramer, L. D. (2007). West Nile virus infection decreases fecundity of *Culex tarsalis* females. *Journal of Medical Entomology*, *44*(6), 1074-1085. https://doi.org/10.1603/0022-2585(2007)44[1074:wnvidf]2.0.co;2

Tandina, F., Doumbo, O., Yaro, A. S., Traoré, S. F., Parola, P., & Robert, V. (2018). Mosquitoes (Diptera: Culicidae) and mosquito-borne diseases in Mali, West Africa. *Parasites and Vectors*, *11*, 467. https://doi.org/10.1186/s13071-018-3045-8

Taylor, R. M., Hurlbut, H. S., Dressler, H. R., Spangler, E. W., & Thrasher, D. (1953). Isolation of West Nile virus from *Culex* mosquitoes. *The Journal of the Egyptian Medical Association*, *36*(3), 199-208.

Turell, M. J., O'Guinn, M., & Oliver, J. (2000). Potential for New York mosquitoes to transmit West Nile virus. *The American Journal of Tropical and Medicine and Hygiene*, *62*(3), 413-414. https://doi.org/10.4269/ajtmh.2000.62.413

Vakali, A., Beleri, S., Tegos, N., Fytrou, A., Mpimpa, A., Sergentanis, T. N., Pervanidou, D., & Patsoula, E. (2022). Entomological surveillance activities in regions in Greece: Data on mosquito species abundance and West Nile virus detection in *Culex pipiens* pools (2019–2020). *Tropical Medicine and Infectious Disease*, *8*(1), 1. https://doi.org/10.3390/tropicalmed8010001

Valiakos, G., Papaspyropoulos, K., Giannakopoulos, A., Birtsas, P., Tsiodras, S., Hutchings, M. R., Spyrou, V., Pervanidou, D., Athanasiou, L.V., Papadopoulos, N., Tsokana, C., Baka, A., Manolakou, K., Chatzpoulos, D., Artois, M., Yon, L., Hannant, D., Petrovska, L., Hadjichristodoulou, C., & Billinis, C. (2014). Use of wild bird surveillance, human case data and GIS spatial analysis for predicting spatial distributions of West Nile virus in Greece. *PLOS One*, *9*(5), e96935. https://doi.org/10.1371/journal.pone.0096935

van den Hurk, A. F., Hall-Mendelin, S., Webb, C. E., Tan, C. S. E., Frentiu, F. D., Prow, N. A., & Hall, R. A. (2014). Role of enhanced vector transmission of a new West Nile virus strain in an outbreak of equine disease in Australia in 2011. *Parasites and Vectors*, *7*, 586. https://doi.org/10.1186/s13071-014-0586-3

Vogels, C. B. F., Göertz, G. P., Pijlman, G. P., & Koenraadt, C. J. M. (2017). Vector competence of European mosquitoes for West Nile virus. *Emerging Microbes and Infections*, *6*(11), 1-13. https://doi.org/10.1038/emi.2017.82

Vora, N. (2008). Impact of anthropogenic environmental alterations on vector-borne diseases. *Medscape Journal of Medicine*, *10*(10), 238.

Wanasen N., Nussenzveig, R. H., Champagne, D. E., Soong, L., & Higgs, S. (2004). Differential modulation of murine host immune response by salivary gland extracts from the mosquitoes *Aedes aegypti* and *Culex quinquefasciatus*. *Medical and Veterinary Entomology*, *18*(2), 191-199. https://doi.org/10.1111/j.1365-2915.2004.00498.x

Xia, H., Wang, Y., Atoni, E., Zhang, B., & Yuan, Z. (2018). Mosquito-associated viruses in China. *Virologica Sinica*, *33*, 5-20. https://doi.org/10.1007/s12250-018-0002-9

Zeidner, N. S., Higgs, S., Happ, C. M., Beaty, B. J., & Miller, B. R. (1999). Mosquito feeding modulates Th1 and Th2 cytokines in flavivirus susceptible mice: An effect mimicked by injection of sialokinins, but not demonstrated in flavivirus resistant mice. *Parasite Immunology*, *21*(1), 35-44. https://doi.org/10.1046/j.1365-3024.1999.00199.x