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Review Article

Overview of West Nile Virus

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ABSTRACT

Flaviviruses had an extensive spread around the universe. West Nile Virus (WNV) is a zoonotic virus that threatens health of humans and animals and causes several epidemics for both hosts. Additionally, it has economic losses due to the expense of treatment of diseased cases, control programs, and animal losses. The epidemic spillover and spreading of the viruses is geographically distinguished in the last few decades. The epidemiological features of WNV are controlled by its insect vectors, their geographical distribution, climatic changes, and changes in arthropods' habitat as a consequence of the change in urbanization behavior as well as the global travel movements. National and international surveillance for WNV transmission is an important strategy to track the virus's spread and effective management methods.

INTRODUCTION

West Nile virus (WNV) is an emerging arthropod-borne pathogen that causes disease in horses and humans, as well as in a variety of other animals. The virus is maintained within the avian wildlife population which acts as a natural reservoir and amplifier for the virus, which is transmitted mostly by mosquitoes **(Gray and Webb, 2014).** According to the world organization for animal health, WNF is considered a notifiable disease that has a serious impact on livestock and public health. West Nile Virus (WNV) belongs to Genus Flavivirus. The genus comprised different health-threatened viruses including Japanese encephalitis virus (JEV), Zika (ZIKV), Yellow fever virus (YFV), Mosquito-borne dengue virus (DENV), Tick-borne encephalitis virus (TBEV), Usutu virus (USUV), and West Nile virus (WNV) (**Pierson and Diamond, 2018; Roehrig, 2013).** In Egypt, *Culex antennatus* is the major mosquito species that is responsible for WNV transmission cycle. Around the world, more than 300 bird species may act as vertebrate hosts for WNV **(Selim et al. 2020).**

The current study aims to present an in-depth update on WNV, concentrating on virus biology and pathobiology, economic importance, epidemiology, diagnostics, prevention, and control. We discuss and review data collected over the previous decade, as well as propose future study directions.

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Epidemiology

The epidemic spillover and spreading of the Flaviviruses is geographically distinguished in the last few decades **(Colpitts et al. 2012).** For instance, WNV and ZIKV were widespread, especially in the western population with a large recorded number of humans infected **(Carlson et al., 2022; Pierson and Diamond, 2020)** Moreover, some WNV outbreaks have been also reported before in France, Italy, Greece, South Africa, Hungary, southeast Romania and the USA **(Bakonyi et al. 2013).**

Furthermore, WNV was detected in several countries in the Middle East and Asia such as Jordan, Palestine, Israel, Iran, Saudi Arabia, and Turkey **(Azmi et al. 2017; Shahhosseini et al. 2017).** Regarding Egypt, WNV infection was reported for the first time in 1950 in north Cairo, then many outbreaks have been documented between 1952 and 1954 **(Eybpoosh et al. 2019).** Human WNV seroprevalence was reported in Egypt and WNV was isolated from both sentinel chickens and mosquitoes in cohort study sites indicating the active circulation of the WNV in the country **(Soliman et al. 2010).** Recently, a serological survey for WNV was performed and the virus was detected in equids in the northern Egyptian Governorates, mostly in Qalyubia and Kafr El Shiek **(Selim and Abdelhady, 2020).**

Based on their nature as arthropod-borne viruses are affected by climatic changes which affect the future of the emergence of zoonotic viral diseases. Climate change is causing rising global temperatures and unpredictable precipitation patterns, both of which are contributing to the spread of mosquito-borne arboviruses and the mosquito populations that transmit them **(Calle-Tobón et al. 2021).** Some potentially have a high risk of the zoonotic side as they may have new hosts which ultimately affect human health **(Carlson et al. 2022).** Subsequently, the epidemiological features of arboviruses especially Flaviviruses may be affected by climatic changes, insect vectors, their geographical distribution, and changes in arthropods' habitat as a consequence of the change in urbanization behavior as well as the global travel movements **(Pierson and Diamond, 2020).**

Economic importance

West Nile Virus (WNV) is an essential emerging zoonotic virus that causes disease in humans, horses, and several species of birds. As for the equine sector, WNV affects the equine industry worldwide due to its clinical outcomes and repercussions on the trade of horses for sale, breeding, and competition. Hence, there is potential for significant economic through death and illness in horses. Horses are particularly affected by WNV and up to 30% of those showing clinical signs may die. Economic impacts were estimated taking into account several aspects, such as vaccination costs, medical and hospital costs, costs for home care, compensation paid for the death of an animal, and costs associated with work absenteeism **(Humblet et al. 2016).** Refering to the birds, some infected bird species may also have reduced survival. There are concerns that species vulnerable to fatal infection may be more prone to extinction, although there is no evidence of this currently. The disease can result in negative perception and therefore unnecessary destruction of wildlife. Effects on wildlife and zoological collections can have a significant impact on tourism (*National Wildlife Health Center | U.S. Geological Survey*, n.d.).

In humans, WNV infection is considered a serious zoonotic disease. Most people (80%) bitten by an infected mosquito show no signs or symptoms. Only around 20% of the people who become infected will develop flu-like symptoms and about 1% will suffer from a severe neurological infection of the virus (encephalitis, meningitis and acute paralysis). Viral illness in humans can result in economic losses due to the cost of treatment and time lost from normal activities **(Barber et al. 2010; Gray and Webb, 2014).**

Virus Classification and genomic organization

The virus is an RNA *Flavivirus* that belongs to the family *Flaviviridae*. The virus is enveloped, spherical-in shape, with an 11 kb positive -sense single-stranded RNA genome. The

translated viral polyproteins consist of three structural (C, prM/M, and E) besides seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) **(Richter et al. 2017).** Based on the genetic and phylogenetic analysis, WNV has two lineages; Lineage 1 and Lineage 2. Lineage 1 includes three sub-lineages a, b, and c, and lineage 1a is circulated in Africa, America, Europe, and the Middle East whereas, Lineage 1b (known as Kunjin virus (KUNV)) is spread in Australia, and Lineage 1c is mostly distributed in India. The sub-Saharan African strains belong to WNV Lineage 2. Other possible WNV lineages have been previously reported such as Lineage 3 (Rabensberg virus) in the Czech Republic and southern Moravia, Lineage 4 in Russia, Lineage 5 in India, and Lineage 6 (Koutango virus) which is isolated from Africa **(Bondre et al. 2007; Cardinale et al. 2017; Lwande et al. 2014).** Serological diagnosis for WNV was preferred to detect the viral infection. WNV antibodies can be detected in domestic animals using a variety of serological procedures, including enzyme-linked immunosorbent assays (ELISA), immunofluorescence assays (IFA), and viral neutralization tests (VNT) **(Beck et al. 2013)**

Virus life cycle and transmission

Based on the world organization for animal health, WNF is an arthropod-borne notifiable disease that has a serious impact on livestock health and public health (*West Nile Fever - WOAH - World Organisation for Animal Health*, n.d.). The virus is an essential emerging zoonotic virus that is maintained in a bird-mosquito-bird transmission cycle and may be accused of disease in humans, horses, and several species of birds as depicted in Figure 1. The virus is naturally maintained in the Culex. This ornithophilic mosquito feeds on birds including C. univittatus and C. pipiens where the former is considered a virus vector while the latter is the virus reservoir. Various passerine birds are considered amplifier hosts which can develop sufficient serum viremia which efficiently infects mosquitoes upon feeding behavior **(Komar et al. 2003).** It could be replicated in birds and mosquitoes which are considered a source of infection to dead-end hosts horses and humans **(Selim and Abdelhady, 2020)** Infection is often asymptomatic or moderate in humans, but in infected horses, symptoms can vary from mild ataxia and muscular weakness to severe ataxia and recumbency and as they developed low-level serum viremia, they are unlikely to infect mosquitoes **(Domanović et al. 2019).**

Within the bird-Culex cycle and the increase of infected mosquitoes, the risk of human infection by mid to late summer. Furthermore, warm temperatures correlate with an increased human incidence within the national or regional parameters. Meanwhile, the increased temperatures can shorten the incubation period of the virus to the infectiousness in mosquitoes and increases viral transmission efficiency to birds, both critical factors for arboviral amplification **(Danforth et al. 2015; Kilpatrick et al. 2008).** Humans can be infected with the virus via the mosquito bites. Blood transfusions **(Pealer et al. 2003)** and organ transplantation are counted from the ways of the virus transmission ways **(Nett et al. 2012).**

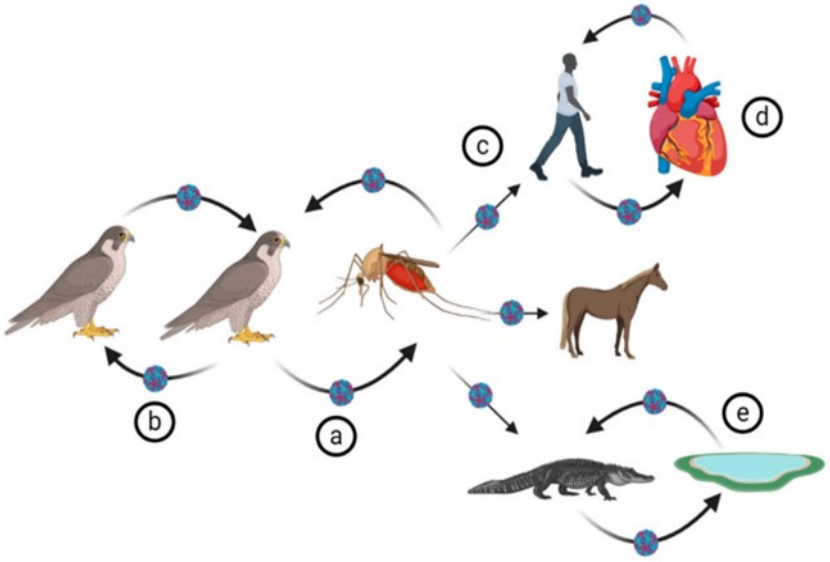

Figure 1: West Nile Fever Virus lifecycle and transmission. (a) WNV maintenance between birds (reservoir) and competent mosquito vector, (b) WNV transmission via direct between birds in commercial farm setting, (c) WNV transmission to various hosts (human, horse and crocodile) via mosquito bite, (d) WNV transmission via blood transfusion and organ transplant in human, (e) WNV infection in crocodile through WNV contaminated water (Habarugira et al., 2020).

Pathogenesis and Clinical Symptoms

At the site of the bite, the salivary materials of the mosquito were inoculated to modulate the initial infection by targeting the keratinocytes and the dendritic cells by suppressing the immune effector cell trafficking **(Lim et al. 2011; Schneider and Higgs, 2008).** Then, the infected dendritic cells and keratinocytes migrated to the lymph nodes, and viremia was induced, the infection spreads to visceral organs and potentially to the central nervous system (CNS), the virus is neurotropic which generating neuroinvasive disease in humans is the virus' capacity to gain access to the central nervous system with neuroinvasive effect. The neuroinvasive mechanism of WNV is implied of i) direct viral crossing of the blood-brain barrier through the cytokine-mediated increased vascular and endothelial permeability; ii) a Trojan horse mechanism in which infected tissue macrophages are trafficked across the blood-brain barrier; and eventually iii) retrograde axonal transport of the virus to the central nervous system via infection of olfactory or peripheral neurons **(Cho and Diamond, 2012).**

The incubation period of WNV is ranged from 2 to 14 days but is prolonged up to 21 days among immunocompromised patients **(Rhee et al. 2011).** The intensity of the viral infection with fever from mild infirmity lasting a few days to a debilitating illness lasting weeks to months **(Zou et al. 2010).** West Nile meningitis is characterized by abrupt onset of fever and headache along with meningeal signs and photophobia. Some patients sometimes developed a coarse tremor, particularly in the upper extremities. Moreover, the virus may develop a virus–associated paralysis due to the destruction of the anterior horn cells of the spinal cord **(Leis and Stokic, 2012).**

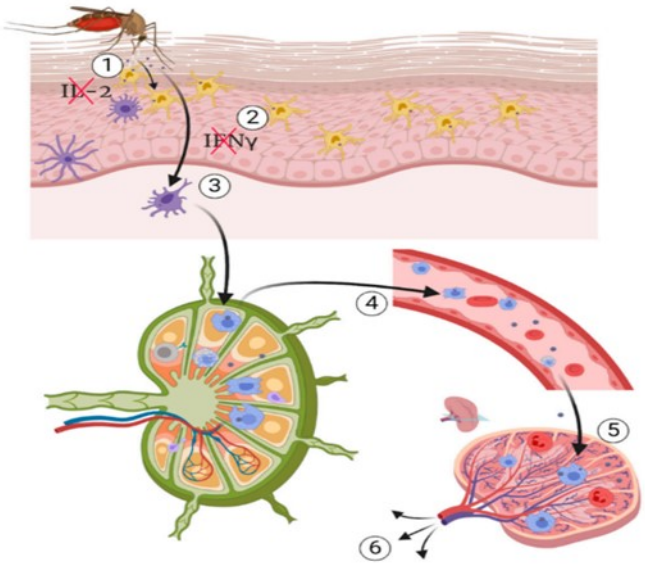

Figure 2: Pathogenesis of WNV infection. (1) Culex quinquefasciatus transmitting WNV during a blood meal on the susceptible host and releasing its infectious saliva, (2) immunomodulation by mosquito's saliva followed by infection of keratinocytes and Langerhans cells, (3) migration of infected cells to nearby draining lymph nodes, (4) viremia followed by migration of infected macrophage from the lymph nodes, and (5) spleen from which the virus spread to other organs of tropism **(Petersen et al., 2013).**

Diagnosis

Under clinical settings, the diagnosis of WNV infection is generally based on the clinical examination, laboratory testing and post-mortem examination. However, the limitation of the clinical diagnosis is that there is no pathognomonic clinical sign of the disease in any affected species. Moreover, in non-endemic areas, clinical examination is less reliable in animals, as the clinical presentation would suggest other infectious diseases with WNV not necessarily being on top of the differentials. In addition, the clinical diagnosis is in most cases presumptive; in humans, it should be supported by travel history. In areas where diseases of similar clinical manifestations are endemic such as malaria, WNV infection might not be on the list of differentials given similar clinical picture, epidemiological information, and mode of transmission (**Habarugira et al. 2020).** The laboratory-based diagnostic approaches comprise of virus isolation, RT-PCR, serology, and pathological examination. Serologically, the diagnosis is based on the detection of IgM and IgG antibodies against WNV. The commercial IgM ELISA kit is used for the detection of anti-prE IgM antibodies in serum samples and the detection of IgM antibodies indicates recent infection and WNV circulation. On the other hand, West Nile Competition Multi-species ELISA Kit was used to detect anti-pr-E IgG in multiple species **(Zeller and Schuffenecker, 2004; Ziegler et al., 2013).** These antibodies can be detected 3–7 days post -exposure. Particularly, IgM can persistently be detected for up to two years, notably in horses, limiting their usefulness in a diagnostic context **(Tardei et al. 2000).** However, the virus neutralizing test (VNT) against WNV remains the gold standard test, as it has high specificity and not only detects the neutralizing antibodies to the virus but also quantifies the neutralizing titers. This assay has some limitations in that it takes a week to get the results and is relatively expensive; hence, its restricted use as a diagnostic tool. The most commonly used molecular diagnostic techniques include reverse transcription polymerase chain reaction (RT-PCR), quantitative RT-PCR (qRT-PCR), and in situ hybridization qRT-PCR has an advantage over regular RT-PCR of quantifying the viral genome. The quantitation is achieved through monitoring the accumulation of double-strand DNA using DNA intercalating fluorescent dyes

such as SYBR® Green. Instead, the quantitation can be achieved by monitoring the amplification of specific target sequences using detection probes **(Sun, 2010).** PCR primers should target NS5, the most conserved genome region in nearly all flaviviruses **(Moureau et al. 2007).**

The molecular diagnosis of WNV targets the E protein region, conserved across several WNV strains (**Berthet et al. 1997).** Being very sensitive, RT-PCR may detect the viral RNA from animals vaccinated with the killed WNV vaccine **(Klenk et al. 2004);** therefore, while screening individuals vaccinated with such vaccines, PCR should be complemented with other diagnostic methods such as virus isolation. Alternatively, the RT-PCR should target WNV-NS5 to segregate viral RNA from the vaccine from replicating virus from infection. The testing should be done on serum or cerebrospinal fluid (CSF) samples.

Prevention and control strategies

There have been some attempts to develop a vaccine against WNV in humans, but to date, there is no approved commercially available vaccine for use in humans **(Dayan et al. 2013).**

Several vaccines are approved for use in horses to aid in the prevention of viremia and clinical disease from West Nile virus infection **(Long et al. 2007).** Since WNV outbreaks in animals precede human cases, the establishment of an active animal health surveillance system to detect new cases in birds and horses is essential in providing early warning for veterinary and human public health authorities (*West Nile Virus*, n.d.). Control strategies and dissemination of information are required to look for WNV in mosquitoes and monitoring for bird deaths and suspicious illness in people and horses to identify areas where WNV is present before it becomes a threat to humans. Biological control of flaviviruses such as WNV has been investigated. This is an indirect technique of control that involves lowering vectors with various biological agents. Bacillus thuringiensis serotype israelensis (Bti), a mosquito larvicide, is the most often utilized agent **(Ben-Dov, 2014; Dambach et al. 2014).** Given the peri-domestic ecology of some mosquito vectors, individuals should strive to eliminate any mosquito breeding site. Additionally, people should remove any stagnant or standing water, as well as any equipment or locations where water may build **(Habarugira et al. 2020).** Avoiding human and horse exposure to WNV-infected mosquitoes remains the cornerstone for preventing WNV disease. Pesticides have also been commonly used to control vectors of various disease vectors. Insect repellent should be used on the skin when exposed to mosquitoes and avoid being outdoors from dusk to dawn when mosquito vectors of WNV are abundant in an endemic area. Of insect repellents recommended for use on skin, those containing *N*,*N*-diethyl-m-toluamide (DEET), picaridin (KBR-3023), or oil of lemon eucalyptus (*p*-menthane-3,8 diol) provide long-lasting protection. Both DEET and permethrin provide effective protection against mosquitoes when applied to clothing. Persons' willingness to use DEET as a repellent appears to be influenced primarily by their level of concern about being bitten by mosquitoes and by their concern that DEET may be harmful to health, despite its good safety record. To prevent transmission of WNV through blood transfusion, blood donations in WNV-endemic areas should be screened by using nucleic acid amplification tests. Screening of organ donors for WNV infection has not been universally implemented because of concern about rejecting essential organs after false-positive screening results. Pregnant women should avoid exposure to mosquito bites to reduce the risk of intrauterine WNV transmission **(Capobianchi et al. 2010).**

CONCLUSION

With the present trend of globalisation and climate change, flavivirus infections have become a worldwide health issue as well as a public health concern. These dangers must be addressed via the collaboration of many stakeholders, including medical professionals, health workers, anthropologists, environmentalists, ecologists, veterinarians, and farmers, among others, utilizing a one-health approach. Furthermore, the discovery of cross-protective vaccinations would be an important step forward in the control and

maybe the elimination of harmful flavivirus infections.

Future prospectives

WNV illness will most certainly remain a public health problem for the foreseeable future; the virus has established itself in a wide range of ecological situations and is transmitted by a reasonably large number of mosquito species. Over the next few years, research activities may be divided across various areas. Research on innovative strategies to limit mosquito exposure in humans and horses is critical and can help avoid other mosquito-borne diseases. This should involve developing novel strategies for reducing mosquito abundance, creating new repellents, and doing behavioral research to improve the usage of existing effective repellents and other personal protective measures against mosquito bites. Understanding the dynamics of no mosquito-borne transmission is critical for preventing vertical transmission between dams and offspring, as well as recipients of blood transfusions and donated organs. known preventative efforts, such as the distribution of knowledge and goods for personal mosquito protection and the deployment of known procedures for lowering mosquito abundance in populations at risk of WNV transmission, must be rigorously applied. National and worldwide surveillance for WNV transmission will be necessary to track the virus's spread and the effectiveness of management methods. Finally, more studies into the ecological drivers of WNV transmission, such as climatic conditions and reservoir and vector population dynamics, might aid in identifying geographic locations at higher risk for WNV illness

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