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REVIEW ARTICLE

West Nile Virus: Molecular epidemiology, pathogenesis, and global public health challenges

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ABSTRACT

The West Nile virus (WNV), belonging to *Flaviviridae*, is an RNA arbovirus and a member of the Japanese encephalitis virus serocomplex, and has materialized as a major growing health risk. Because of its swift geographic expansion and development of severe diseases, a small proportion of infected cases progress to neuroinvasive disease, tied to high fatality rates, mainly in geriatric and immunocompromised populations. The enzootic transmission of WNV occurs between avian hosts and Culicine mosquitoes, with humans and various mammals functioning as accidental terminal hosts. Molecular epidemiology has identified at least seven genetic lineages, with lineages 1, 2 and 5 responsible for human disease, and considerable phylogenetic diversity influenced by migratory bird dispersal. Acute flaccid paralysis, meningitis, encephalitis, fever, and long-term neurologic sequelae are among the clinical symptoms. Diagnosis relies on serological detection of WNV-specific IgM and confirmatory neutralization assays, though cross-reactivity with related flaviviruses complicates interpretation. Currently, no licensed human vaccines or specific antiviral therapies exist, and management remains supportive. Preventive strategies focus on vector control, blood donor screening, and personal protective measures. Advances in molecular virology, ecological surveillance, and vaccine development are improving understanding of viral transmission and pathogenesis. However, knowledge gaps remain regarding vector competence, host susceptibility, and predictive modelling of outbreaks. A multidisciplinary approach integrating genomic, ecological, and clinical data is essential to guide effective prevention, therapeutic innovation, and global preparedness against WNV.

Keywords: Encephalitis, Equines, *Flaviviridae*, JEV serocomplex, WNV

A. Introduction

A part of the Japanese encephalitis virus serocomplex, the West Nile virus (WNV) is an RNA virus that is carried by arthropods and belongs to the genus *Flavivirus* and family *Flaviviridae*[1]. Since its discovery in Uganda in 1937, WNV has proliferated throughout Africa, the Middle East, Australia, and the Americas, emerging as the most common virus carried by mosquitoes in temperate climate[2] (Table 1).

Transmission occurs primarily through Culicine mosquitoes, with birds serving as the main amplifying hosts[3]. Most infections are asymptomatic, but approximately 20% manifest as a self-limiting fever, and less than 1% develop neuroinvasive conditions such as acute flaccid paralysis, encephalitis or meningitis, which carry significant morbidity and mortality[4]. Molecular studies reveal several distinct viral lineages, of which lineages 1 and 5 are responsible for human and animal disease in India, and lineages 1 and 2 worldwide[5]. Various animals, including wildlife, domestic and companion animals, can be affected by the disease. When infected, most animals, including horses and humans, act as dead-end hosts owing to low levels of viraemia[6]. Most infections in horses and humans are asymptomatic with varying clinical manifestations, often involving nervous system functions. In other animals like bovines, ovines, camelids, canines and reptiles, WNV induces the formation of antibodies[7, 8].

The earliest serological evidence of West Nile virus infection in India was documented in Bombay in 1952, followed by confirmation in the South Arcot district of Tamil Nadu in 1982[9]. Over the subsequent three decades, recurrent outbreaks of WNV were reported predominantly from the southern states, including Tamil Nadu, Karnataka, and Andhra Pradesh[10]. Since 1974, serological investigations have demonstrated the presence of West Nile virus in animals across multiple regions of India[10]. Antibodies against WNV were

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Table 1. Historical perspective of West Nile Virus spread

Period/ Year	Key Event	Location	Significance
1937	First isolation of West Nile virus from a febrile woman	West Nile district, Uganda	Discovery of WNV; initially considered a mild dengue-like febrile illness[15, 16]
1950s	Ecological studies of virus transmission	Upper Nile region, Africa	Established Culex mosquitoes–bird transmission cycle; birds identified as amplification hosts and humans as dead-end hosts[17]
Late 1950s	First neurological disease observations	Egypt and Israel	Early evidence that WNV can cause neurological manifestations[18]
1960s–1980s	Sporadic outbreaks and equine cases	Africa, Europe, the Middle East	WNV is largely underestimated, as most infections were mild or asymptomatic[19, 20]
1970s–1980s	Identification of related virus variants	Australia	Discovery of Kunjin virus, a subtype/variant of WNV[21]
1996–1997	Large outbreak with neuroinvasive disease	Romania	Major turning point; high morbidity and mortality in elderly[22]
1999	First appearance in the Americas (NY99 strain)	New York City, USA	Major encephalitis outbreak in humans; high mortality in birds and horses[23]
2000–2003	Rapid geographic spread across North America	USA	Virus spread coast-to-coast by 2003 [24]
2000s	Expansion to other regions	Canada, Caribbean, Mexico, South America	Establishment of WNV across the Western Hemisphere[24, 25]
2000s	Development of surveillance systems	USA	ArboNET was established for monitoring human, avian, and mosquito infections[13]
2010s	Endemic establishment and emergence of lineage 2 in Europe	Hungary, Greece, Italy, Spain	Demonstrated spread of previously Africa-restricted lineage[20]
2012	Major outbreak in the USA	United States	One of the largest epidemics since its introduction in 1999[26]
2020s	Climate-change influenced transmission	North America and Europe	Extended mosquito season and expanded geographic range[27]
2025	Early seasonal cases detected	New York City, USA	Continued endemic circulation with positive mosquito pools across boroughs[10,28]

first detected in ardeid birds, including pond herons (*Ardeola grayii*) and cattle egrets (*Bubulcus ibis),* in Andhra Pradesh in 1981[10]. Subsequent studies confirmed viral circulation in both ardeid and terrestrial bird populations in Karnataka, as well as among wild resident and migratory avian species in eastern and northern parts of the country[11]. WNV-specific antibodies have also been identified in domestic pigs from Karnataka and Punjab, as well as in chickens from a poultry farm in Assam[12]. In Karnataka, pigs have been utilized as sentinel animals for monitoring WNV circulation. Longitudinal studies indicated that once pigs seroconverted to Japanese encephalitis (JE) and WNV, they retained immunity for up to three years[13]. Despite advances in surveillance and understanding of viral ecology, no licensed human vaccine or specific antiviral therapy exists, making prevention strategies reliant on vector control, personal protection, and public health monitoring[14].

Considering the increasing prevalence and emergence of WNV as an important neurotropic arbovirus, with potential for misdiagnosis in regions of co-existence of multiple flaviviruses, a comprehensive understanding of

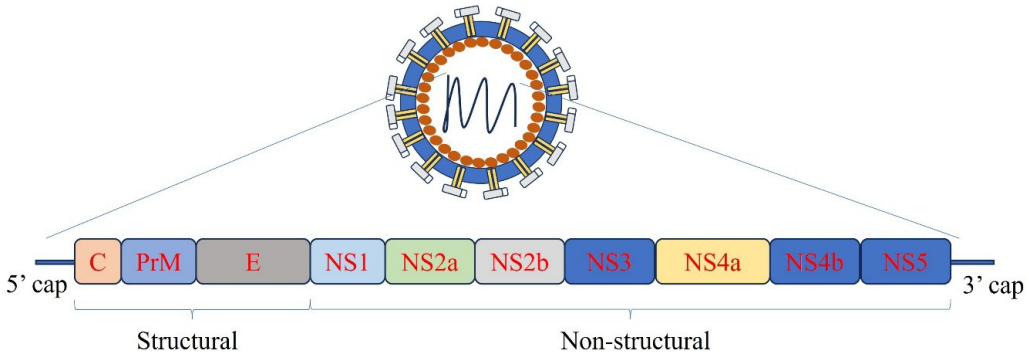
public health significance of the disease and epidemiology is essential. This review, therefore, aims to underscore and summarize the current knowledge on epidemiology, transmission, pathogenesis, clinical manifestations, diagnostic challenges and control strategies from an Indian perspective, while at the same time highlighting the knowledge gaps that need further investigations.

B. Major Outbreaks of the Disease

Historically, several outbreaks of West Nile Virus (WNV) have been recorded, with major epidemics occurring in Israel (1951) and later in Romania, Tunisia, Russia, Canada, Greece, Algeria, the Czech Republic, and the Congo between 1994 and 1999[29, 30]. In animals, major outbreaks have primarily occurred among birds, particularly corvids and equines, although evidence of infection have been reported in over 35 animal species, most presenting asymptomatically. Notably, an outbreak of equine encephalitis attributed to WNV was recorded in Italy in 1998, affecting 14 horses and resulting in 4 deaths[31]. Another outbreak in France in 2003 resulted in several human cases and five equine meningoencephalitic cases, with a reported seroprevalence of 34% in equines[32]. North America has evidenced continuous outbreaks of WNV since 1999, affecting corvids, wild and exotic birds and equines, with thousands of avian mortalities and hundreds of equine cases documented so far[31]. In India, the prevalence of WNV has shown an upward trend, with serological evidence of infection in equines around Pune and 1,096 equines from five states. Additionally, serological evidence of WNV infection in pigs and frugivorous bats around Chandigarh suggests potential spillover into domestic and wild animals. In avian populations, neutralizing antibodies have been detected from pond herons, cattle egrets in southern parts of the country, as well as migratory and resident waterbirds from eastern and northern regions of the country[33].

C. Structure and Morphology of the Virus

The Virus's genome comprises single-stranded, positive-sense RNA with an approximate length of 11 kbp and codes for three structural and seven non-structural proteins, which are synthesised as a single polyprotein comprising around 3000 amino acids[3]. The virions are enveloped with icosahedral symmetry and approximately 50 nm in size. The structural proteins required for the formation of complete virions include capsid protein (C), pre-membrane protein (PrM) and envelope protein (E). The non-structural proteins include NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5, of which NS1 and NS2A are required for the processing of viral polyprotein along with NS2B, which acts as a cofactor for NS3 protease[34]. The NS1 protein also promotes neurodegeneration by promoting β-amyloid deposition in the CNS[35]. The NS5 protein, a methyltransferase, acts as RdRp (RNA-dependent RNA polymerase) and suppresses interferon signalling in anti-viral immune responses[36]. Whereas other non-structural proteins are small, hydrophobic and perform disparate functions[37]. All NSPs act together and facilitate viral replication, pathogenesis and immune evasion and are promising targets for drug design and development[38] (Figure 1).



C	Capsid
PrM	Pre-membrane
E	Envelope

NS1	Replication/ evasion
NS2a	Replication
NS2b	Protease-cofactor
NS3	Protease/ helicase
NS4a	Membrane remodelling
NS4b	Immune evasion
NS5	Polymerase

Figure 1. Pictorial representation of the structure and genome of WNV

D. Molecular Epidemiology

Advances in genomic sequencing and global surveillance have substantially refined the phylogenetic classification of West Nile virus[18]. Current evidence indicates that WNV can be divided into at least seven distinct genetic lineages throughout the world, defined by nucleotide sequence divergence of approximately 25–30%[3]. Of these, only lineages 1 and 2 have been consistently associated with clinically significant mammalian and avian disease. In India, lineages 1 and 5 are predominant[18]. Importantly, phylogenetic clustering does not always align with the geographic distribution of the virus. This discordance is largely attributable to the long-distance dispersal of WNV through migratory avian hosts, which facilitates the intercontinental spread of genetically distinct strains[39]. Ongoing genomic surveillance continues to refine lineage boundaries and provides critical insights into viral evolution, epidemiology, and the emergence of strains with enhanced virulence or altered transmission dynamics.

Lineage 1

West Nile virus Lineage 1 exhibits a broad geographic distribution, encompassing North and Central America, Africa, Europe, Asia, Australia, and the Middle East[40]. Phylogenetic analyses subdivide lineage 1 into three major sublineages (1a, 1b, and 1c). Sublineage 1a is most often encountered and has been detected in Africa, Europe, the Middle East, Asia and the Americas, including the strain responsible for the 1999 New York outbreak (NY99). This sublineage has been implicated in virtually all major human encephalitis outbreaks concerning WNV worldwide, including the ongoing epidemics in North America. In 2011, sublineage 1a WNV was first reported in India, where it was detected from the serum of a febrile patient during an outbreak of neuroinvasive disease, further underscoring its pathogenic potential and global spread. Sublineage 1b, historically referred to as Kunjin virus (WNV-KUN), is endemic to Australia and likely extends into Papua New Guinea and parts of Southeast Asia[41]. Human disease caused by WNV-KUN is relatively uncommon; however, a notable 2011 equine outbreak in south-eastern Australia highlighted its ability to cause severe disease in horses under favourable ecological conditions. Sublineage 1c has thus far been reported exclusively from India[42]. However, recent phylogenetic studies suggest that isolates previously classified within sublineage 1c exhibit sufficient divergence to warrant reclassification as a distinct lineage, now proposed as lineage 5[43]. Newer Russian and Egyptian isolates, namely Ast01-182 and Eg101, have been isolated from Madurai, Tamil Nadu, which belong to lineage 1 of WNV[18].

Lineage 2

Historically, WNV lineage 2 was considered largely restricted to sub-Saharan Africa, where it was primarily associated with sporadic human infections manifesting as mild febrile illness and was not typically linked to large outbreaks or severe disease[43]. However, since the mid-2000s, the epidemiological profile of lineage 2 has shifted dramatically. Emerging evidence from Russia, Hungary, Italy, and Greece has demonstrated that lineage 2 strains are capable of causing severe neuroinvasive disease in humans, as well as fatal infections in birds and horses[44]. These findings established lineage 2 as an important cause of morbidity and mortality in both humans and animals, challenging the earlier perception of its limited pathogenicity and underscoring its expanding role in WNV epidemiology in Europe.

Lineage 5

The WNV lineage 5 mainly includes Indian isolates obtained from 13 different human cases showing genetic divergence from lineages 1, 2, and 3 (24-25% for lineage 1 and 2; 24-25% from lineage 3). Moreover, cross-neutralization studies employing lineage-1 strain Eg-101 specific polyclonal antibodies against Indian isolates suggest substantial antigenic variation[45].

E. Ecology

An enzootic transmitting cycle between a large number of mosquito vectors and a diverse range of avian hosts (more than 200 species) sustains the West Nile virus in nature[46]. Although representatives of the genus *Culex* are known to be the main vectors of WNV, over 60 different species of mosquito have been linked to the virus's spread[47]. Particularly significant in both North America and Europe is the *Culex pipiens* complex, which includes *Cx. pipiens pipiens*, *Cx. pipiens molestus*, and *Cx. Quinquefasciatus*[48]. The potential for local transmission is influenced by the physiology, behaviour, and ecological distribution of each species, as these factors determine vector competence, host preference, and population abundance.

Because the majority of Culicine mosquitoes are ornithophilic, or bird-feeding, they serve as effective enzootic vectors, maintaining the spread of viruses throughout bird populations. However, their frequent proximity to humans, especially in urban environments, also enables their role as epidemic vectors, bridging transmission from birds to humans. This is facilitated by the widespread presence of immature mosquito stages in aquatic habitats associated with artificial structures. Urban water management practices—such as the creation of wastewater treatment systems and conservation wetlands—may further alter vector and reservoir host communities, thereby modifying local WNV risk[49] (Figure 2).

F. Pathogenesis

The most frequent cause of West Nile virus infection is epidermal inoculation of viral material through

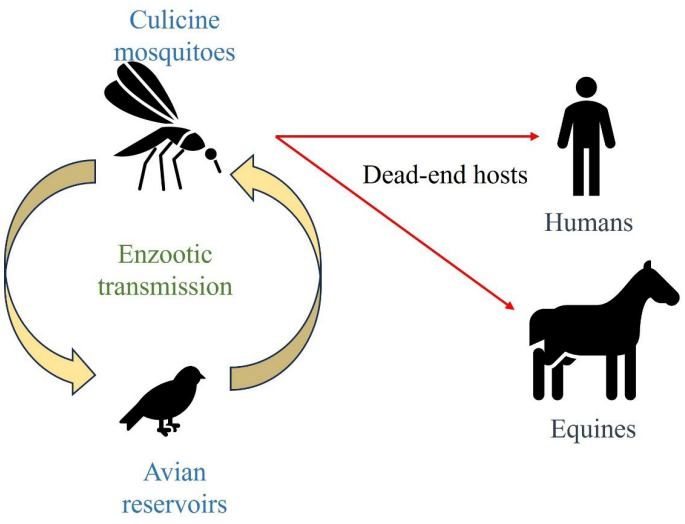


Figure 2. Representation of the transmission cycle of WNV

mosquito bites. Following entrance, the virus first replicates in dendritic cells (which are antigen-presenting cells) and epidermal keratinocytes at the injection site[38]. It then spreads to regional lymph nodes, where further replication occurs before disseminating into the bloodstream and subsequently infecting visceral organs[50,51]. In susceptible individuals, the virus may ultimately invade the central nervous system (CNS).

The early host response is mediated by the innate immune system. Infection induces production of proinflammatory cytokines, particularly type I interferons (IFNs), which play a critical role in restricting viral replication[52]. Additional mediators, including complement, chemokines, tumour necrosis factor (TNF), interleukin-1β, and innate cell-mediated responses, further contribute to viral control[53]. However, excessive or prolonged pro-inflammatory signalling can also cause immunopathologic damage. Viral clearance typically requires activation of the adaptive immune response, in which both humoral (B cell–mediated) and T cell–mediated immunity are necessary for limiting viral replication and preventing sustained inflammation-induced injury. Several mechanisms have been proposed for WNV neuroinvasion, including axonal transport along olfactory or peripheral neurons, cytokine-mediated disruption of the blood–brain barrier, migration of infected immune cells across endothelial tight junctions (the so-called "Trojan horse" mechanism), and direct infection of endothelial cells. Once within the CNS, WNV exhibits neurotropism with a predilection for extrapyramidal structures, including the brainstem, basal ganglia, thalami, and cerebellum; infection of neurons in these regions leads to cell death and contributes to the clinical manifestations of neuroinvasive disease[54]. The virus uses conserved receptors to enter the cells, which include neuronal $\alpha v\beta 3$ integrin and heparan sulphate[55]. The viral ligand E (envelope) glycoprotein binds the receptors, and internalization occurs by clathrin-mediated endocytosis and is recognised by TLR 3/7/9 along with other PRRs and leads to IFNAR signalling[56]. Furthermore, the immunopathological damage to neurons occurs by overactivation of NMDA receptors, leading to Ca2+ influx in neurons and causing neuronal death by excitotoxicity following virus-mediated astrocyte damage[57] (Figure 3).

G. Clinical Presentation

Most West Nile virus infections are asymptomatic (~80%), and approximately 20% of cases present as a self-limited febrile illness, while 0.4–0.7% progress to

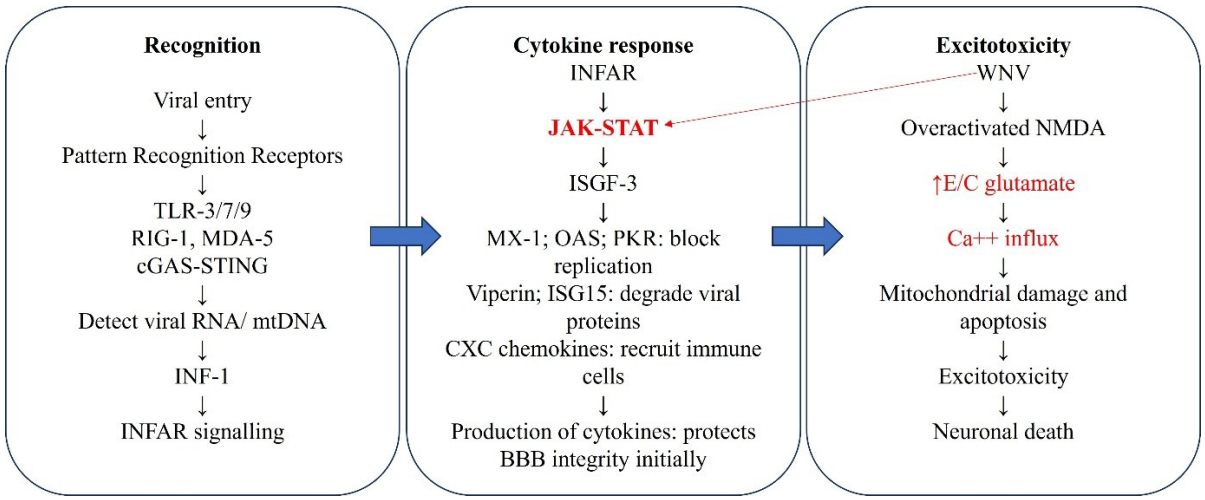


Figure 3. Molecular pathogenesis and immunopathological damage mechanism in the CNS by WNV

neuroinvasive disease[58]. The incubation period following mosquito exposure is typically 2–6 days (up to 14 days), though it may be prolonged in immunocompromised hosts, particularly transplant recipients and transfusion-related cases[59].

Neuroinvasive disease occurs in approximately 20% of horses infected with the virus and is characterised by fever and ataxia, including stumbling, incoordination, and staggering. Additional signs include limb paralysis, muscle fasciculations, weakness, prostration, defective proprioception and rarely acute death[60]. Among the horses developing neurological disease, 30% may die (CFR), and ~20 recover with neurological deficit. Clinical history, prevalence of mosquitoes, location and vaccination status of horses can support initial suspicion for the disease[60].

H. Diagnosis

West Nile virus infection should be considered in the differential diagnosis of patients presenting with febrile illness or neurologic symptoms during the summer and fall in endemic areas, particularly when there is a history of mosquito exposure, recent blood transfusion, or organ transplantation. Because clinical features overlap with other viral and bacterial infections, laboratory confirmation is essential[61]. The samples to be collected for diagnosis in case of suspicion for WNV include blood, serum and CSF from live animals and brain tissue from dead horses with a clinical history of severe neurological disease[60]. The signs of neurological disease often overlap with other neuroinvasive diseases like rabies, alphaviral equine encephalitis, Japanese encephalitis, etc. Hence, differential diagnosis with these diseases is also a necessity[60].

Serologic Testing

The principal and most sensitive method for diagnosing WNV infection in immunocompetent individuals involves detecting WNV-specific immunoglobulin M (IgM) antibodies in serum and/or cerebrospinal fluid (CSF). This is generally achieved through the use of IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) or a duplex microsphere-based immunoassay technique[62]. WNV-specific IgM antibodies are detectable in >98% of patients by day 8 of illness; therefore, if initial IgM testing performed early in the course of infection is negative, repeat testing after day 8 is recommended[58]. Detection of WNV-specific IgM in non-bloody CSF is considered diagnostic of central nervous system (CNS) infection, as IgM does not ordinarily cross the intact blood–brain barrier[63].

However, interpretation of IgM results requires caution because antibodies may persist long after acute infection. Longitudinal studies have demonstrated persistence of serum IgM in 23–60% of patients for more than 16 months post-infection, and CSF IgM may remain detectable for up to 6 months[64]. Among viraemic blood donors, the average time to IgM negativity was approximately 5 months. For this reason, a positive IgM result does not necessarily distinguish acute infection from past exposure[65].

IgG testing alone is not diagnostic of acute disease, as it indicates prior infection with WNV or antigenically related flaviviruses. To confirm recent infection and exclude cross-reactivity with other flaviviruses (e.g., St. Louis encephalitis virus, Japanese encephalitis virus, Murray Valley encephalitis and Usutu virus), plaque reduction neutralization testing (PRNT) for WNV-specific neutralizing antibodies may be performed. PRNT remains the gold standard for serologic confirmation, though it is available only through reference laboratories such as the U.S. Centers for Disease Control and Prevention (CDC) and select state public health laboratories[62].

Molecular Testing

Detection of viral RNA by reverse transcription polymerase chain reaction (RT-PCR) is limited by the short duration of viremia, which typically resolves before the onset of neurologic disease in immunocompetent individuals. Sensitivity also varies by specimen type. In a cohort of 105 serologically confirmed patients with acute WNV disease, the sensitivity of RT-PCR was 26% for serum, 20% for plasma, and 17% for CSF[66]. Thus, RT-PCR is not considered a first-line diagnostic tool in the general population. Molecular testing, however, plays a critical role in immunocompromised patients who may not mount an antibody response. For example, individuals receiving B-cell–depleting monoclonal antibodies (e.g., rituximab, ocrelizumab) or transplant recipients are more reliably diagnosed with RT-PCR or metagenomic sequencing of serum, plasma, or CSF[58]. These molecular methods may be more sensitive than serology in such settings.

Tissue-Based Diagnosis

In certain cases, particularly postmortem evaluations, WNV infection can be confirmed by immunohistochemical staining (IHC) or RT-PCR detection of viral RNA in tissue samples. Histopathologic findings in neuroinvasive disease include microglial nodules, neuronal necrosis, and perivascular lymphocytic inflammation, predominantly affecting the brainstem, basal ganglia, thalami, and cerebellum. Patients with meningitis or meningoencephalitis may additionally exhibit leptomeningeal inflammation[67].

Rapid diagnostic platforms

Rapid diagnosis is essential in imparting the required treatment and also in surveillance and control of infections, especially in endemic areas. Several methods of diagnosis have been developed for rapid, pen-/

bed-side diagnosis of WNF, including Rapid Analyte Measurement Platform and chemiluminescent assays. These methods most commonly use serum or CSF as preferred diagnostic samples but can also be adapted to CNS tissue homogenates and cell-culture supernatant[68, 69].

I. Treatment and Prevention

Clinical management remains supportive, focusing on the stabilization of vital functions, mitigation of secondary brain injury, and long-term neurorehabilitation for patients with neuroinvasive disease. Investigational therapies—including interferon-α, ribavirin, immunoglobulins, and RNA-based strategies—have demonstrated antiviral activity in vitro or in animal models, but translation to human clinical efficacy has been inconsistent or absent[70]. High-titre immunoglobulins (e.g., Omr-IgG-am) and monoclonal antibodies show promise, yet their effectiveness in controlled human trials remains inconclusive. Similarly, RNA-targeted therapies (siRNA, antisense oligomers) and small-molecule inhibitors of viral protease or replication demonstrate preclinical efficacy but face challenges in timing of administration and CNS penetration[71].

Preventive strategies currently rely on mosquito vector control, personal protective measures, and blood safety screening. While effective equine vaccines exist, no human vaccine has been licensed. Several platforms—including inactivated, live-attenuated, chimeric, DNA, mRNA, and virus-like particle (VLP) vaccines—are in preclinical or early clinical phases, underscoring ongoing global efforts toward prophylaxis[72].

Critical gaps

The primary obstacles in WNV therapeutic development arise from both biological and logistical factors. First, the narrow therapeutic window presents challenges, as viremia often resolves by the time patients develop neuroinvasive disease, limiting antiviral efficacy[16]. Second, the blood–brain barrier restricts drug delivery to sites of neuroinflammation[73]. Third, the sporadic and geographically unpredictable nature of outbreaks hinders recruitment for large-scale clinical trials[74]. Additionally, most affected patients are elderly or immunocompromised, complicating trial design and therapeutic response evaluation.

Nevertheless, research suggests that early intervention with antibody-based therapies, RNA-targeted agents, or interferons could be effective if administered during the viraemic phase. Advances in nanoparticle delivery systems and host-targeted therapies may improve CNS penetration and broaden the therapeutic window[75]. Furthermore, preclinical data on monoclonal antibodies targeting the WNV envelope protein highlight their dual role as potential post-exposure prophylaxis and therapeutic agents[76].

The successful deployment of equine vaccines suggests technical feasibility; however, prioritization has been limited by the episodic nature of human outbreaks compared with other flaviviruses such as dengue, yellow fever, or Japanese encephalitis[77].

Translational gaps

Future research on West Nile virus must address the persistent translational gap between promising preclinical findings and demonstrable human efficacy. Despite substantial progress in animal models, the successful application of these discoveries in equine and human populations has been limited, largely due to the sporadic nature of outbreaks and the complexity of the virus's ecology. To bridge this divide, innovative approaches are required across trial design, therapeutic development, vaccine research, vector control, and integrated surveillance.

One key area of focus is clinical trial design. Traditional trial frameworks are poorly suited to an infection characterized by unpredictable and geographically variable outbreaks. Adaptive and platform trial models, deployed across endemic regions, could improve the efficiency and feasibility of evaluating both therapeutics and vaccines. Such designs would allow multiple interventions to be tested simultaneously, while also providing the flexibility to adapt as epidemiological conditions evolve.

Therapeutic strategies remain another critical frontier. Monoclonal antibody therapies, which have shown protection in preclinical studies, must now advance into controlled human trials. Similarly, novel approaches such as small interfering RNA (siRNA) and antisense oligomers require optimization to ensure effective delivery across the blood–brain barrier, one of the central challenges in treating WNV neuroinvasive disease[78]. Host-directed antivirals represent an additional avenue, with the potential to enhance innate immune defences while minimising the risk of immunopathology.

Vaccine development remains a high priority. Accelerated testing of DNA, mRNA, and chimeric vaccine platforms is urgently needed, particularly given the rapid advancements achieved during the COVID-19 pandemic[79]. Leveraging these established technologies may allow for scalable, cost-effective vaccines capable of providing durable protection in at-risk populations. Determining correlates of protection, such as neutralizing antibody titres and cellular immune responses, will be crucial for guiding both vaccine development and deployment strategies.

Finally, the adoption of the One Health approach is essential. Because WNV circulates at the intersection of human, animal, and environmental health, surveillance systems must integrate human clinical data with

veterinary and ecological monitoring. Strengthened cross-sectoral networks will be critical for identifying hotspots, predicting outbreak risk, and guiding targeted interventions such as vaccine deployment and intensified vector control.

Immunization and disease control measures

Currently, no vaccine is approved for human use; however, four vaccines are licensed for equine protection. WN InnovatorTM, Vetera WNV, and Prestige® WNV are three of these formulations that are whole-virus inactivated, while RecombitekTM Equine WNV is a live chimeric vaccination that expresses the prM/E genes in a canarypox vector[80]. All vaccines, except Vetera WNV, which is derived from the E159 equine isolate, are based on the NY99 strain. These vaccines have been shown to elicit protective immunity in horses, with efficacy lasting approximately one year[41].

Interestingly, an experimental study in mice reported that oral administration of vancomycin, neomycin, ampicillin, or metronidazole aggravated disease severity in multiple flavivirus infections[81]. These findings underscore the need for further well-designed, controlled investigations to establish effective therapeutic strategies for WNV. Prevention remains the primary strategy for controlling West Nile virus infections. The development of robust early warning systems and the implementation of targeted vector control programs are critical for reducing spillover transmission to humans. Effective entomological surveillance can provide advance indication of potential outbreaks, enabling timely intervention[82]. Immediate control measures should focus on reducing vector populations through the strategic use of organophosphate or synthetic pyrethroid insecticides. Recently, the adoption of the 'One Health' approach has gained prominence, promoting coordinated actions across human, animal, and environmental health sectors, as the virus circulates among humans, birds, horses, and mosquitoes, integrated surveillance systems are essential for early detection of viral activity in avian or equine populations which often precede human cases, and incorporating these signals into predictive models could provide vital early warnings. Research into climate change, avian migration patterns, and biodiversity shifts is also necessary to understand how ecological changes influence viral amplification and spillover into human populations. In Europe, this approach has enhanced the promptness of blood safety measures and facilitated the timely execution of vector control strategies[83].

Vector control remains a cornerstone of WNV prevention, but traditional methods such as larvicides, source reduction, and adulticides have not consistently prevented large-scale outbreaks. Research is needed to refine entomological surveillance, including the integration of molecular xeno-monitoring techniques that detect WNV RNA in mosquito pools as early outbreak indicators. Innovative biological control methods, such as the introduction of *Wolbachia*-infected mosquitoes, an entomopathogenic fungus, or larvivorous fishes like Gambusia, hold promise but require careful ecological risk assessments[80]. Genetic modification of mosquito populations through CRISPR-based gene drives, or sterile insect techniques (SIT), represents another frontier in vector control, though these approaches raise important ecological and ethical considerations[84]. Advances in mathematical modelling, which combine climate data, land-use patterns, and mosquito population dynamics, could further enhance the precision of outbreak prediction and improve targeted vector management.

Environmental management offers additional opportunities. Modifying urban water storage, improving sewage management systems, and managing wetlands can reduce breeding habitats, while public health campaigns can encourage behavioural changes that limit human–mosquito contact. Personal safety precautions, such as applying repellents, protective clothing, and avoidance of peak mosquito activity, remain effective, though research is needed to enhance compliance and long-term adoption.

Finally, the absence of nationwide equine vaccination and the licensed human vaccines highlight a critical prevention gap. Accelerated development and evaluation of DNA, mRNA, and chimeric vaccine platforms represent a promising research frontier. Integration of vaccination, once available, into a One Health–based prevention framework that coordinates human, veterinary, and ecological health responses will be essential for reducing the burden of WNV[85].

J. Future Perspectives

Future efforts to combat West Nile virus will focus on bridging the gap between existing preventive strategies and innovative interventions. In the short term, strengthening integrated surveillance systems that combine human, veterinary, and ecological data will be critical for early outbreak detection and targeted responses. Advances in vector control, including precision biological methods, genetic modification of mosquito populations, and climate-based predictive modelling, hold promise for reducing transmission but require rigorous ecological and ethical evaluation.

In the longer term, the development of a human vaccine remains the most important research priority. Novel platforms such as mRNA, DNA, and chimeric vaccines could accelerate progress, building on lessons from COVID-19 vaccine development. Parallel efforts in therapeutics, including monoclonal antibodies, RNA-based antivirals, and host-directed therapies, may offer new treatment options for neuroinvasive disease, which

currently lacks effective interventions. Finally, adopting a One Health framework that integrates environmental management, wildlife monitoring, and human health measures will be essential for sustainable control. As climate change, urbanization, and ecological shifts continue to expand the geographic range of WNV, multidisciplinary and globally coordinated research will be central to reducing its long-term public health impact[85].

K. Conclusion

West Nile virus remains an important emerging threat to global public and animal health. Classifying its epidemics into historical and contemporary phases provides valuable insight into its epidemiological patterns. As an emerging global pathogen, it also serves as a model for the international public health community to enhance communication, enhance collaborative networks, and improve preparedness strategies against presently neglected and future infectious disease threats. Strengthening diagnostic capacity in veterinary laboratories, promoting awareness among veterinarians and implementation of integrated vector control measures are crucial in mitigating the impact of virus. Furthermore, continued research on host-vector dynamics, reservoir patterns, and regional epidemiology will be essential in improving preparedness and prevention of future outbreaks affecting both human and animal populations.

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