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REVIEW ARTICLE ON WEST NILE FEVER

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ABSTRACT

West Nile virus (WNV) is a neurotropic human pathogen that is the causative agent of West Nile fever.Geographically and genetically it is a diverse virus.The Initial isolation of WNVis in West Nile district of Uganda in 1937,from a woman. WNV become an important cause of human and animal disease worldwide.Occasionally, this virus infects other vertebrates also and causes fever. They are an enveloped virus of the genus *Flavivirus*.In nature it is maintained in an enzootic cycle between mosquitoes andbirds. Vector for natural transmission of WNVare themosquitoes. They alsocause sporadic outbreaks of disease in horses as well as in humans. So it may results in encephalitis, meningitis, febrile illness and flaccid paralysis.Most of the human infections with WNV are asymptomatic, severe neurological disease may develop, thus resulting in long term sequelae or even death. In 1957, the first outbreak of this neuroinvasive disease caused by WNV was reported among the elderly in Israel.This review updates the most recent information about Virology, Life cycle, Transmission cycle, Diagnosis, Prevention, and Treatment etc.

INTRODUCTION

West Nile fever is an infection caused by the West Nile virus, which is mainly spread by mosquitoes [1,2]. This infectious disease can cause flu-like symptoms [3]. Virus is transmitted among birds through the bite of infected mosquitoes. First appeared in the U.S in 1999. Most people infected with this virus do not show any symptoms or have mild symptoms. The major problem with this infection is that there is no specific prophylaxis or treatment exists against the disease in humans [4].

WEST NILE VIRUS [5]

VIROLOGY

- Genus : Flavivirus
- Family : Flaviviridae
- Enveloped in positive single stranded RNA(ssRNA) virus
- One of the Japanese encephalitis antigenic serocomplex of virus.
- Consists of 8 phylogenetic lineages, but only lineage 1 and 2 are associated with disease in humans.
- Replicate very fast andspread more easily to birds at higher temperatures. Climate change affects the epidemiology of this disease.

STRUCTURE

- Enveloped virus with icosahedral symmetry.
- Envelope: Outer layer of the virus is a lipid bilayer
- Image reconstructions &cryoelectron microscopy reveals a 45–50 nm virion, which is covered with a relatively smooth protein shell.

- From the envelope of the virus spiky glycoproteinsproject out. These proteins help the virus to bind the cells of its host.
- The protein shell is made of two structural proteins. They are the glycoprotein E and the small membrane protein M.
- Protein E has many functions including receptor binding, viral attachment and entry into the cell through membrane fusion.
- The outer protein shell is covered by a host derived lipid membrane,that is the viral envelope.
- The lipid membrane of flavivirus contains cholesterol and phosphatidyl serine but other elements of the membrane have yet to be identified.
- The role of lipid membrane in viral infection includes :

  a) Acts as signalling molecules.

  b) Enhances the entry into the cell.

  c) Cholesterol plays an integral part in WNV entering a host cell.The two viral envelope proteins E and M are inserted into the membrane.

- Capsid proteins are structural proteins having 105 amino acid residues. They are the first proteins created in an infected cell. Its function is to pack RNA into the developing virus. They prevent apoptosis by affecting AKt pathway [signal transduction pathway that promotes survival and growth in response to extracellular signals].
- The RNA genome is bound to capsid (C) proteins to form the nucleocapsid.

The nucleocapsid is made up of two parts, that is:

- First part is the protein shell also known as the capsid. This structure protects the substance inside.
- The second part of the nucleocapsid is genetic information in the form of single-stranded RNA[8].

GENOME

WNV is a positive sense, single-stranded RNA[ssRNA] virus. Its genome is approximately 11,000 nucleotideslong and is flanked by 5' and 3' non-coding stem loop structures. The coding region of the genome codes for3 structural proteins and7 non-structural (NS) proteins, proteins that are not incorporated into the structure of new viruses. The WNV genome is first translated into a polyprotein and later cleaved by virus and host proteases into separate proteins (i.e. NS1, C, and E).

NON STRUCTURAL PROTEINS

Consist of NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. These proteins mainly assist viral replication or act as proteases.The non-structural proteins are located near the 3' end of the genome.

LIFE CYCLE

- WNV attaches to the host cell at an unknown receptor site andentered to the cell through the endocytosis process. If the virus is in the inside of the lysosome host cell, then the fusion of the viral and host cell membranes occurs.
- Nucleocapsid gets deposited into the cell cytoplasm. Then the ssRNA get dissociates from the capsid and converts to a polyprotein. This translation or conversion of polyprotein occurs at the endoplasmic reticulum. Also occurrence of envelope proteins being translocate across the membrane and into the ER lumen, while other remaining products enter to the cytoplasm.
- Immediately after the formation of RNA – dependent RNA polymerase and helicase, they get released from the polyprotein. Then the RNA replication begins. Synthesis of (-) strands from the (+) RNA template also occurs. Replication process takes place in the cytoplasm. As capsid proteins are made, they associate with the nucleic acid to form a nucleocapsid.

- Thus envelope proteins accumulate in the membrane and assemble in a way so that they mask the surface protein that originally allowed the membranes to fuse upon viral entry.
- The newly formed nucleocapsid then associates with ER membrane containing the surface proteins and then an assembled virion buds into the ER lumen. The virus then exists the cell through the host secretory path[9,12,18].

TRANSMISSION

In case of humans, infection occurs as a result of bites from infected mosquitoes. Mosquitoes become infected when they feed on infected birds, which circulate the virus in their blood for a few days. The virus eventually gets into the mosquito's salivary glands. During later blood meals (when mosquitoes bite), the virus may be injected into humans and animals, where it can multiply and cause illness. The virus may also transmitted through contact with other infected animals, their blood, or other tissues [6]. West Nile Virus is most commonly spread to humans by the bite of an infected mosquito. Mosquitoes become infected when they feed on infected birds. Infected mosquitoes then spread West Nile virus to people and other animals by biting them [10]. In a very small number of cases, West Nile virus spread through:

- Exposure in a laboratory setting
- Blood transfusion and organ donation
- Mother to baby, during pregnancy, delivery, or breast feeding [6,20].

West Nile virus is not spread:

- Through coughing, sneezing, or touching
- By touching live animals
- From handling live or dead infected birds. Avoid bare-handed contact when handling any dead animal. While disposing a dead bird, use gloves or double plastic bags to place the carcass in a garbage can.
- Through eating infected birds or animals. Always follow instructions for fully cooking meat from either birds or mammals [6,20].

Usually WNV becomes active in the spring because previously infected, dormant mosquitoes become active during this time or because infected birds migrate during this period of time. The virus is usually passed from infected birds to bird-feeding mosquitoes or from infected mosquitoes to birds. Until late summer, this cycle usually continues. Because all mosquitoes do not feed on humans and birds. In late summers, mosquitoes that feed on both humans and birds reach their annual activity and will pass the virus onto humans. The WNV reproduces itself through the process of conjugation, so there is an exchange of genetic material happens. The WNV being a retrovirus, so it goes through a lysogenic cycle. The incubation period of the West Nile Virus is 2-15 days. The WNV usually cycles around birds and several types of arthropods, but occasionally makes it out of this cycle and reaches humans. The primary arthropods that carry the West Nile Virus are mosquitoes. Basically, the West Nile Virus can infect vertebrates and invertebrates [7,12].

SYMPTOMS

Most people infected with the virus do not have any symptoms or have mild symptoms. Rarely, the virus can enter the brain and cause life-threatening complications. About 1 in 5 people may have mild, flu-like symptoms, which include:

- Mild skin rash
- Joint pains
- Fever
- Headache
- Nausea
- Vomiting
- Diarrhoea
- Body aches
- Drowsiness
- Loss of appetite
- Swollen lymph nodes (lymph glands)
- An achy feeling in the back and muscles

Symptoms usually occur 3 to 14 days after a person is bitten by an infected mosquito. The incubation period is usually 3 to 14 days. They can last a few days up to several weeks. They usually go away on their own [3,13]. About 1 in 150 people who are infected with West Nile virus develop a more severe illness. This happens when the virus enters the brain. There it can cause encephalitis that is inflammation of the brain. It can also cause meningitis. This is inflammation of the membranes that surround the brain and the spinal cord. Symptoms of these illnesses include[10]: A sudden high fever (above 102°F) , Very bad / Severe headache, Stiff neck, Blurry vision or worsening eyesight / vision loss, Feeling disorientated or confused, Tremors or muscle jerks, Seizures / convulsions, Coma, Weakness or partial paralysis, Confusion, Reduced coordination, Fatigue, Muscle weakness , Stupor, disorientation, Numbness

PREVENTION

Prevention and control of WNF require an integrated approach that includes vaccination, enhanced mosquito control and improved clinical management. Preventive tools other than drugs and vaccines can also help to prevent the transmission of the infection by avoiding bites of mosquito responsible for the disease transmission. By minimizing the exposure of skin surface to infected mosquito bites is the main prevention. It is by covering the exposed skin area by wearing long-sleeved shirts and pants, treat clothing and gear and take steps to control mosquitoes indoors and outdoors. The most effective way to prevent infection from West Nile Virus is to prevent mosquito bites during the day and night. Get rid of mosquitoes breeding areas. The elimination of standing water where mosquitoes lay eggs, the installation of window and door screens, the minimization of outdoor activities coincident with the maximum activity of mosquitoes, reporting dead birds to local authorities and supporting mosquito control programs. Use insect repellentcontaining DEET(N,N-diethyl-meta-toluamide) for prevention.DEET is not recommended for children under the age of 2 months. Other insect repellents approved by the CDC include picaridin, oil of lemon eucalyptus or PMD, and IR3535[15,18,22,23].

DIAGNOSIS: West Nile virus can be diagnosed by a number of different tests:

1) WNV Antibody Testing:

- Laboratory diagnosis of WNV is generallydone by testing of serum or cerebrospinal fluid (CSF) received from WNV infected patients at the time of their clinical presentation.
- This test mainly detectsthe presence of WNV-specific Immunoglobulin M (IgM) antibodies by using the IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA). Serum IgM antibody may persist for more than a year.
- Presence of IgM antibodies in the cerebrospinal fluid is indicates infection of the CNS, because IgM antibody does not cross the blood-brain barrier.
- Immunoassays for WNV-specific IgM are available commercially. WNV-specific IgM antibodies can be detected within 3 to 8 days after onset of illness and persist for 30 to 90 days.
- Initially, serological testing was based on IgM antibody capture assays (MAC-ELISA) and in indirect IgG ELISAs, followed by retesting of positive samples by PRNT. Later on, ELISAs using monoclonal antibody blocking assays were set up.
- WNV IgG antibodies are generally detected shortly after IgM antibodies. It persists for many years following a symptomatic or asymptomatic infection. Therefore, the presence of IgG antibodies is the only evidence for previous infection and clinically compatible cases with the presence of IgG, but not IgM, should be evaluated for other etiologic agents.
- IgG antibody sero-conversion (or significant increase in antibody titers) in two serial specimen collected at a one week interval by enzyme-linked immunosorbent assay (ELISA).

- Neutralisation assays are also used for diagnosis.
- Plaque-reduction neutralization tests (PRNTs) performed in some state public health laboratories and CDC. These tests can help to determine the specific infecting flavivirus. It can also confirm the acute infection by demonstrating a fourfold or greater change in WNV-specific neutralizing antibody titer between acute- and convalescent-phase serum samples collected 2 to 3 weeks apart[14,15,16].

2) Antigens Testing:

- Detection of viral antigens is based on antigen-capture ELISAs, dipstick assays, or immune-histo chemical (IHC) methods.
- Viral cultures and tests to detect viral RNA (e.g., reverse transcriptase-polymerase chain reaction [RT-PCR]) assays, quantitative real-time RT-PCR and nucleic acid sequenced-based amplification can be performed on serum, CSF and tissue specimens.
- All these are collected early in the course of illness and, if the results are positive infection canbe confirmed.
- All these assays have been mainly used in mosquito pools, animals and human samples (blood, CFS).
- When virus is unlikely to be present in human, samples are usually collected after the onset of clinical signs and symptoms.
- Immunohistochemistry (IHC) can detect WNV antigen in formalin-fixed tissue. Negative results of these tests do not rule out WNV infection.
- Viral culture, RT-PCR and IHC can be requested through state public health laboratories or CDC.
- All these assays have been extensively used for detection of anti-WNV antibodies in human and animal samples.

Brain tests: Electroencephalography (EEG) measures brain's activity or MRI scan can help to detect brain inflammation [14, 17, 18, 19].

TREATMENT

- Vaccine or specific antiviral treatments for West Nile virus infection are not available for humans.
- But for diseased horses vaccines are available now.
- Over-the-counter pain relievers are used to reduce fever, relieve some symptoms, mild headaches and muscle aches.
- In case of severe infection, patients are often need to be hospitalized for receiving supportive treatment measures, such as intravenous fluids, pain relief medication and nursing care.

Interferon therapy: Scientists are now investigating interferon therapy. It is a type of immune cell therapy used as a treatment for encephalitis caused by West Nile virus. Some research shows that people who receive interferon recover better than those who don't receive the drug, but more study is needed [12,19,20].

RESEARCH TODAY

The main focus of research is to find an effective means forcuring West Nile Virus infection. There is no any current treatment for WNF but scientists are very close to find an effective means for the cure of infection. The study highlights that there are long term health risks involved with the West Nile Virus. The NIAID continues to look into the health effects of the West Nile Virus infection. National Institute of Allergy and Infectious Diseases (NIAID) awarded grants to scientists studying small-molecular weight compounds as potential antivirals to treat flaviviruses including West Nile Virus. Other therapeutic approaches that are being investigated include:

- Monoclonal antibodies that target WNV particles to inhibit spread of infection.
- Monoclonal antibodies to target and destroyWNV-infected cells.
- Broad-spectrum therapeutics for flaviviruses, including West Nile Virus.

Structural Protein	Function
[C]-Capsid Protein	They enclose RNA genome and package RNA into immature virions.
[prM]-Precursor membrane proteins	Viruses with M protein are infectious. The presence of this M protein allows the activation of proteins involved in viral entry into the cell. This protein is present on immature virions, by further cleavage by furin to M protein, the virions thus become infectious.
[E]-Envelope Protein	A glycoprotein that forms the viral envelope, binds to receptors on the host cell surface in order to enter the cell.

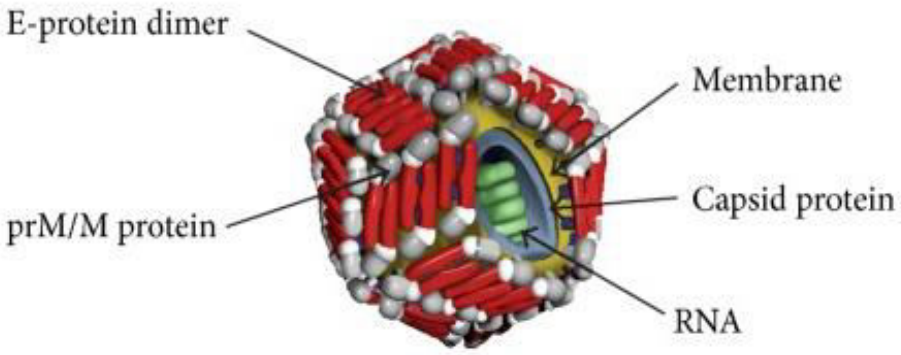
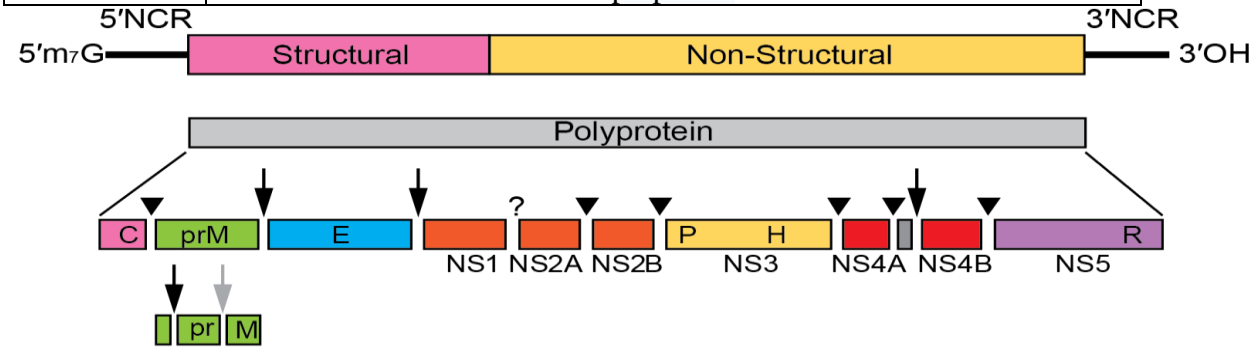


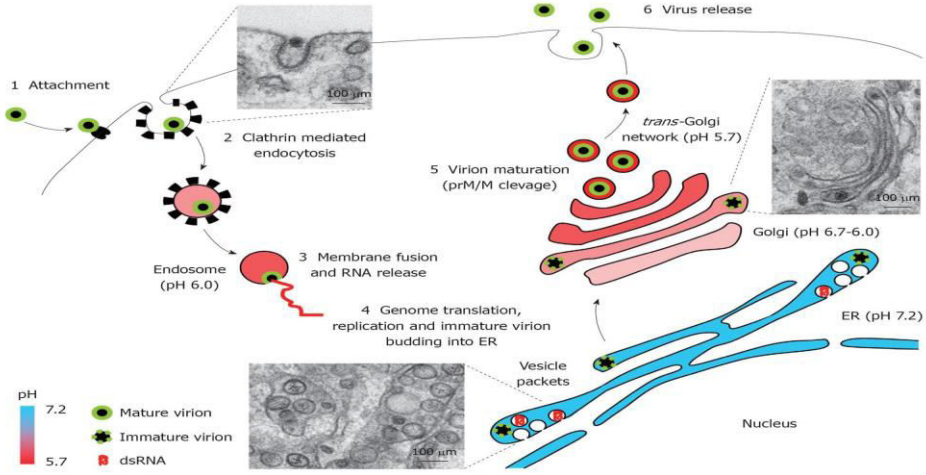
Figure 1: Structure of Wnv Virion [The Global Ecology and Epidemiology of West Nile Virus]

NS1	They act as cofactor for viral replication specifically for the regulation of the replication complex.
NS2A	They are involved in viral replication, virion assembly and inducing host cell death.
NS2B	Act as a cofactor for NS3 and together forms the NS2B-NS3 protease complex.
NS3	A protease responsible for cleaving the polyprotein to produce mature proteins.Also acts as a helicase.
NS4A	A cofactor for viral replication specifically regulates the activity of the NS3 helicase.
NS4B	They inhibit interferon signalling.
NS5	They are the largest and most conserved protein of WNV, NS5 acts as a methyltransferase and a RNA polymerase, But it lacks proofreading properties



- **Figure 2**: FLAVIVIRUS [ICTV Virus Taxonomy]

- **REPLICATION** CYCLE



- **Figure 3**: West Nile Virus Replication Cycle In An Infected Cell [18]

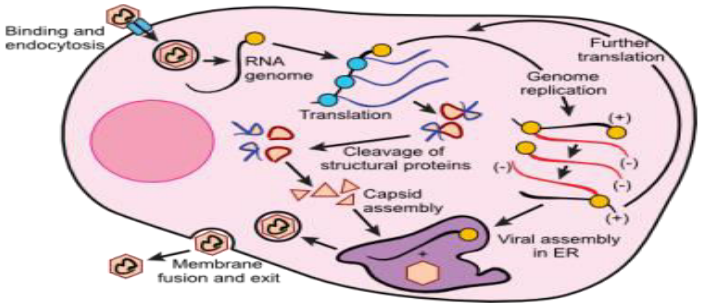


Figure 4: Reproductive Cycle [Https://Flaviviruswestnile.Weebly.Com/Life-Cycle.Html]

TRANSMISSION CYCLE

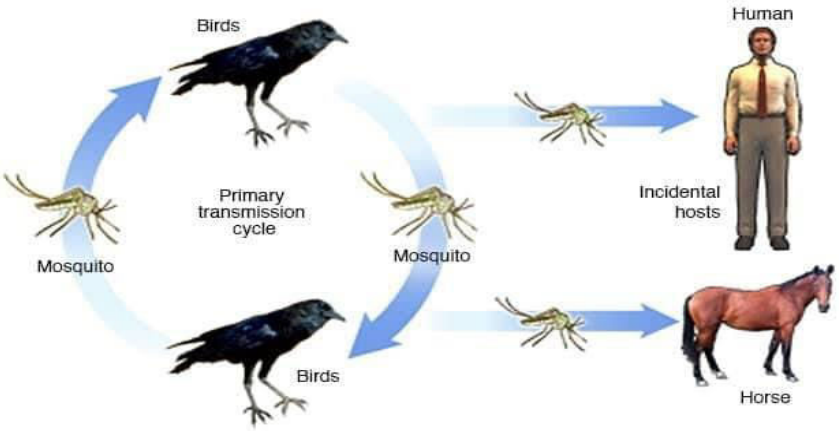


Figure 5: West Nile Virus Transmission Cycle (Https://Www.Mayoclinic.Org/Diseases-Conditions/West-Nile-Virus/Multimedia/West-Nile-Virus-Transmission-Cycle/Img-20006044)

- Therapeutics that is able to cross the blood-brain barrier.
- Identification of drug targets for viruses that infect the nervous system.
- Broad-spectrum immunotherapeutic, such as monoclonal antibodies or bi-specific antibodies development target specific viral Pathways [21].

CONCLUSION

The major risk associated with this infected disease is non-availability of vaccines for curing disease, limited treatment options. Transmission through organ donation and blood transfusion from infected ones is also a major problem. WNV causes human disease, which is West Nile Fever. Weather, climatic (temperature, humidity) change leads to changing ecosystems, change in microbial adaptation, human susceptibility to infection, failure to immunize enough individuals, unscientific township and improper waste disposal may be the reasons for West Nile Fever. Vaccines are available for use in horses but not yet available for humans. Birds are the natural hosts of West Nile Virus.WNV are mainly transmitted to people through the bites of infected mosquitoes. The virus can cause severe disease and death in horses.WNV can cause a fatal neurological disease in humans. Birds are the reservoir hosts of WNV. Use 3Rs for protecting against mosquitoes. They are Reduce, Repel, and Report.

REFERENCES:

- Caren Chancey, Andriyan Grinev, EvgeniyaVolkova, and Maria Rios. The Global Ecology and Epidemiology of West Nile Virus.BioMed Research International. 2014; 2015: 1-20
- Miguel A Martín-Acebes, Juan-Carlos Saiz. West Nile virus: A re-emerging pathogen revisited. World Journal Virology. 2012; 1(2): 51–70.
- https://familydoctor.org/condition/west-nile-virus
- https://www.ecdc.europa.eu/en/west-nile-fever/facts/factsheet-about-west-nile-fever
- West Nile Virus, Wikipedia
- https://www.mayoclinic.org/diseases-conditions/west-nile-virus/multimedia/west-nile-virus-transmission-cycle/img-20006044
- https://study.com/academy/lesson/west-nile-virus-structure-and-function.html
- http://www.microbiologytext.com/5th_ed/Printer/book/displayarticle/aid/492
- Tonya M. Colpitts, Michael J. Conway, Ruth R. Montgomery, ErolFikrig. West Nile Virus: Biology, Transmission, and Human Infection. *Clinical Microbiology Reviews.2012; 25(4: 635-648.)*
- https://flaviviruswestnile.weebly.com/life-cycle.html
- https://www.cdc.gov/westnile/healthcareproviders/healthCareProviders-Diagnostic.html
- Emily M. Ambizas, Alberto H. Ambizas. West Nile Virus-Infectious disease. *US Pharm.* 2012;37(8):31-34.
- Tardei, Evaluation of Immunoglobulin M (IgM) and IgG Enzyme Immunoassays in Serologic Diagnosis of West Nile Virus Infection. Journal of Clinical Microbiology.2000; 38(6): 2232–2239
- Miguel A Martín-Acebes and Juan-Carlos Saiz. West Nile virus: A re-emerging pathogen revisited. World Journal of Virology. 2012; 1(2): 51–70.
- https://www.mayoclinic.org/diseases-conditions/west-nile-virus/diagnosis-treatment/drc-20350325
- Kerala State Pharmacy Council. Pharmacy Council Journal. 2019; 24(2): 23-26.