



## Review Article

### West Nile Virus Infection among Animals and Humans in India

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#### ABSTRACT

Since the introduction of West Nile Virus (WNV) in the New York City of USA in 1999, it has become a global public health concern. The outbreak of WNV has been reported in various parts of the world. The global spread of WNV has indicated that WNV is a re-emerging pathogen. Evidence of WNV infection in India is reported by sero-prevalence in 1952 and subsequently by virus isolation from mosquito, bat and man. Most of the WNV isolates from India belong to Lineage 5, while occasional isolates belonging to lineage 1a have also been reported, which are shown to be more virulent than those of lineage 5 isolates. WNV usually causes mild febrile illness but sometime neuro-invasive disorder has been reported. Currently, there is no licensed vaccine for human beings, although a number of veterinary vaccines have been licensed for horses. The information about prevalence of WNV among animal population in India is scanty. This review attempts to compile and analyze the information on WNV epidemiology, diagnosis and phylogeny from Indian perspective.

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#### INTRODUCTION

West Nile Virus (WNV) is recognized as the most widespread zoonotic mosquito-transmitted arbovirus of public health importance (Pattan et al., 2009). It is a member of the genus *Flavivirus* and belongs to the Japanese encephalitis virus (JEV) antigenic complex under family *Flaviviridae* (De Filette et al., 2012). WNV was first isolated and identified in 1937 from the blood of a woman presented with mild febrile illness in the Nile district of Uganda (Smithburn et al., 1940). Since then many outbreaks of WNV have been reported in different parts of the world such as Egypt (1951), Israel (1957), France (1962–65), South Africa (1974), India (1980–81), Ukraine (1984), Algeria (1994), etc (Murgue et al., 2001). Until 1995, WNV was not considered an important human and animal pathogen as disease occurred only sporadically in humans and horses. However, first large outbreak of WNV was reported in Romania in 1996. The introduction of WNV in New York City of USA in 1999 has raised the major public health and veterinary concerns (Asnis et al., 2000; Roehrig, 2013). The New York 1999 (NY99) strain of WNV was first isolated from a dead American Crow (Lanciotti et al., 1999). It has been reported that 36% of the WNV infected horses either died of WNV or were euthanized. At present, WNV is widely prevalent in Africa, Southern Europe, Russia, Middle East, India, Australia and North America (Brandler and Tangy, 2013). From 1999 to 2010, more than 2.5 million people have been infected, with over 12,000 reported cases of encephalitis or meningitis and over 1,300 deaths (Kilpatrick, 2011). WNV infection is a self-limiting, non-fatal mild febrile illness causing mortality in horses,

domestic and wild birds (Komar, 2000). This virus is known to be active in mosquitoes, birds and pigs (Paramasivan et al., 2003). WNV is reported to be maintained in the nature in a cycle involving birds and mosquitoes. Mosquitoes are principle vectors and birds act as both carriers and amplifying hosts for WNV. Animals and humans act as dead-end host (Paramasivan et al., 2003). In India, WNV infection has been reported from human (Balakrishnan et al., 2013; Chowdhury et al., 2014), animals (Ratho, et al., 1999) and birds (Mishra et al., 2012; Rodrigues et al., 1981). The purpose of this review is to review the status of WNV infection in India, both in human and animal population. The information on WNV epidemiology, diagnosis and phylogeny from Indian perspective has been reviewed in this article.

#### West Nile Virus Structure

WNV is a small spherical, enveloped particle containing single-stranded positive-sense non-segmented RNA genome having icosahedral symmetry. The genome is about 11 kb in size containing a single, long open reading frame flanked by non-coding regions at both ends (Brinton, 2002). Both the 5' and 3' non-coding regions of the genome form stem-loop structures that aid in replication, transcription, translation and packaging (Friebe and Harris, 2010). The viral RNA is translated as a single polyprotein that is post- and co-translationally cleaved by host and viral proteases, resulting in three structural (capsid, envelope and pre-membrane) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) (Figure 1). The surface of virus particles contains two viral proteins; envelope protein

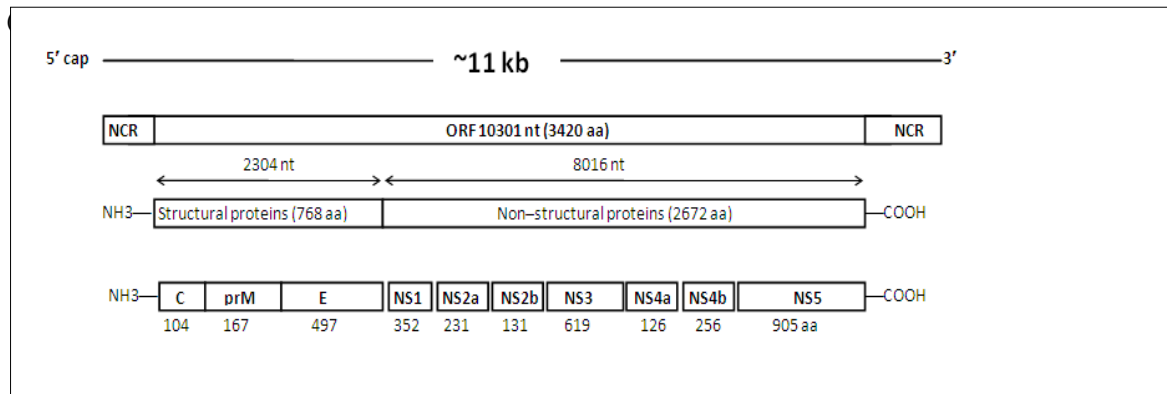


Figure 1. West Nile Virus Genome and Proteins

particles, mediates binding and fusion during virus entry. The M protein (8 kDa), produced during maturation of nascent virus particles within the secretory pathway, is a small proteolytic fragment of the precursor prM protein (18–20 kDa). Capsid protein (12 kDa) is involved in packaging of the viral genome and forming the nucleocapsid core (Mukhopadhyay et al., 2005).

Most neutralizing antibodies against flaviviruses recognize E protein. Crystallographic analysis of the soluble ectodomain of flavivirus E protein has revealed three domains (Modis et al., 2003; Rey et al., 1995). Domain I (DI) is the central domain and consists of an eight-stranded  $\beta$ -barrel that participates in the conformational changes associated with the acidification in the endosome. Domain II (DII) contains 12  $\beta$ -strands and is formed from two extended loops that project from DI. DII is involved in the acid-catalyzed type II fusion event. The putative fusion peptide, which mediates insertion into the target cell membrane, is located at the tip of domain II distal to the

transmembrane region. DIII adopts a seven-stranded immunoglobulin (Ig)-like fold and exposes peptide loops with a role in cellular receptor binding and is a major target of neutralizing antibodies (Brinton, 2014).

Non-structural protein 1 (NS1) has both a “cellular” form and a “secreted” form; highly immunogenic and plays role in replication (Westaway et al., 2002). NS3 is a large (70 kDa) multifunctional protein having protease, nucleoside triphosphatase (NTPase) and helicase activity. This protein is responsible for cleaving other non-structural proteins from the viral polyprotein. The NS5 is a large (103 kDa) highly conserved multifunctional protein having RNA-dependent RNA polymerase (RdRP) and a methyltransferase (MTase) activity; it is necessary for viral replication (Lindenbach and Rice, 2003). Other non-structural proteins like NS2A, NS2B, NS4A and NS4B inhibit the component of innate immune response against virus infection and thus play a role in virus replication.

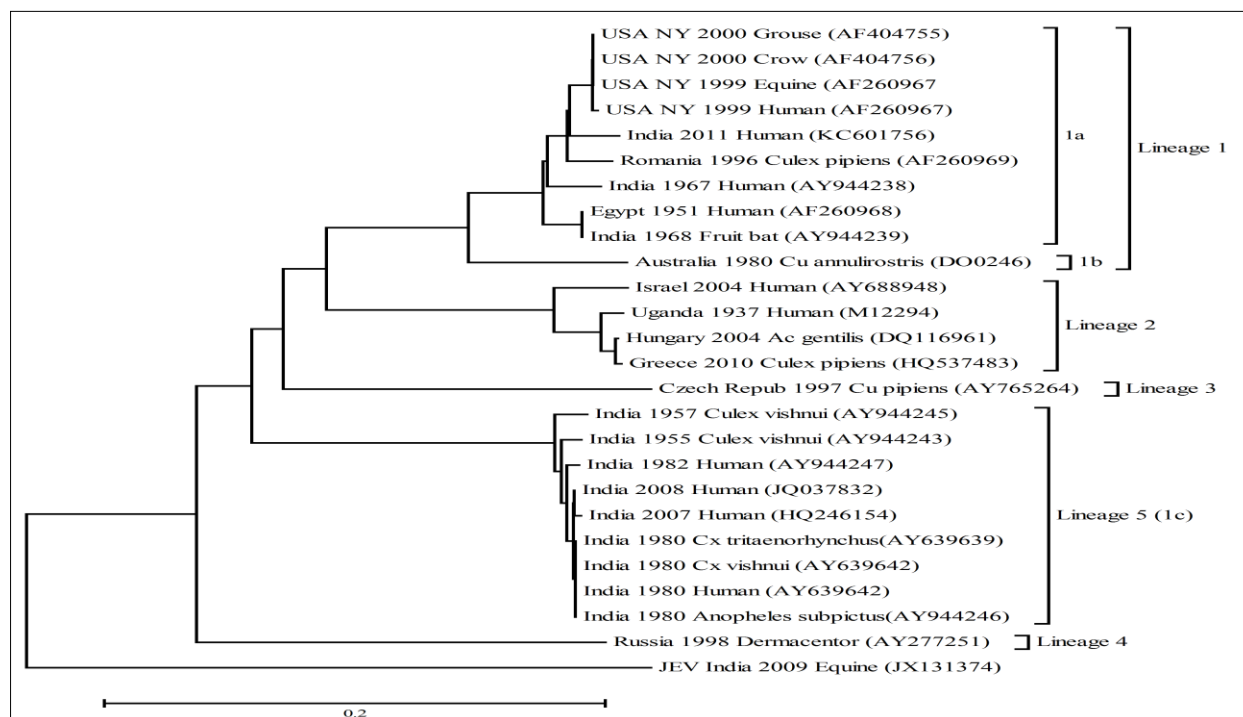


Figure 2: WNV phylogeny based on sequence of C-prM-E region showing five distinct lineages of WNV

### WNV Phylogenetic Diversity

Based on phylogenetic analysis, WNV has been previously classified into two major genetic lineages (Kramer et al., 2008). Lineage 1 is widespread in Europe, the USA, the Middle East, India, Africa and Australia while lineage 2 is mainly present in Sub-Saharan Africa, Madagascar and Central and Eastern Europe (Bakonyi et al., 2006; Platonov et al., 2008). In general, lineage 1 viruses are considered to be more virulent than the lineage 2 viruses. Lineage 1 is further subdivided into three different clades: 1a, 1b and 1c (De Filette et al., 2012). WNV-1a is mainly found in Europe, North America, Middle East and Africa (May et al., 2011). WNV-1b includes the Australian Kunjin virus and lineage 1c contains some Indian isolates.

Subsequently, based on sequence of C-prM-E region, five lineages of WNV have been established (Figure 2). Lineage 3 is represented by single virus strain isolated from mosquitoes in the Czech Republic, designated the Rabensburg virus (Bakonyi et al., 2005) and lineage 4 includes WNV isolated from *Dermacentor marginatus* ticks in the north-west Caucasus Mountain valley (Lvov et al., 2004). WNV strains from India belonging to a clade 1c of lineage 1 have now been classified as lineage 5 (Bondre et al., 2007). Three more putative lineages have been described. The Sarawak Kunjin virus strain is significantly different to the other Kunjin viruses and proposed as lineage 6, while the African virus (Koutango) has been proposed as lineage 7

(De Filette et al., 2012). WNV detected in *Cx. pipiens* mosquitoes captured in Spain have been proposed as lineage 8 (Vazquez et al., 2010).

### WNV Epidemiology

The natural life cycle of WNV involves transmission from mosquitoes (primarily of the genus *Culex*) to wild birds (Figure 3). Birds act as amplifying host of the virus and the WNV has been isolated from nearly 300 species of birds. Mosquitoes become infected when they feed on infected birds that have high levels of viraemia (Pfeffer and Dobler, 2010). The virus then infects and replicate in the midgut of the mosquito (McGee et al., 2010; Richards et al., 2012). After replication in the midgut epithelium, the virus reaches the salivary glands from where it is transmitted to mammalian hosts during feeding (Colpitts et al., 2012; Suthar et al., 2013). Vertical transmission of WNV from female *Culex pipiens* mosquitoes to their progeny has been demonstrated in the laboratory (Bugbee and Forte, 2004). Transovarial transmission of WNV has also been experimentally demonstrated in *Cx. vishnui* mosquito (Mishra and Mourya, 2001). WNV can also be transmitted through blood transfusion, organ transplantation, breast-feeding and trans-placental transmission (Sampatkumar, 2003).

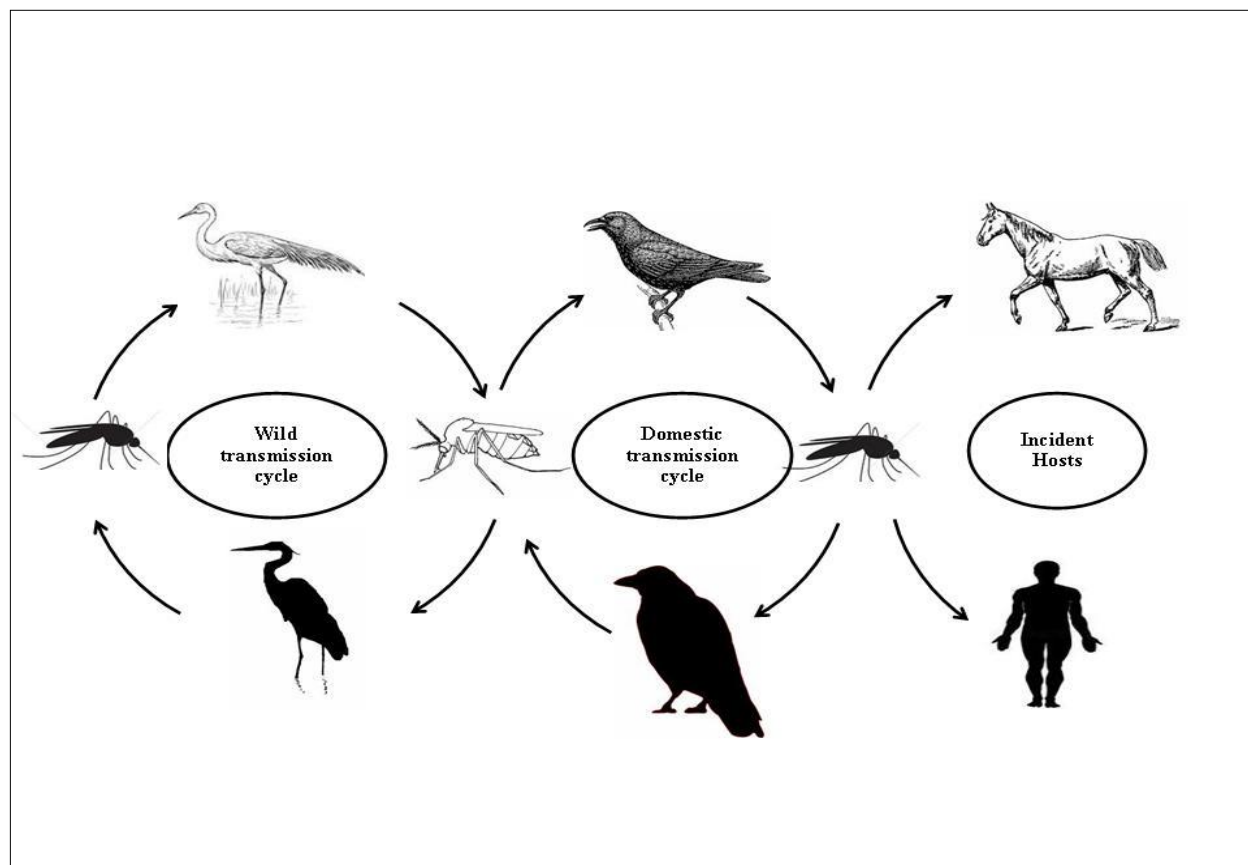


Figure 3: West Nile Virus Transmission Cycle

Ornithophilic mosquito species act as vectors for transmission of infection from viraemic birds to vertebrate hosts. The infected mosquito species vary according to geographical area. *Culex* mosquitoes are accepted as the primary global transmission vector (Hamer et al., 2008; Reisen et al., 2006; Vitek et al., 2008). There are reports of WNV transmission by *Aedes* mosquitoes, though they are not considered a primary vector in nature (Cupp et al., 2007; Farajollahi and Nelder, 2009; Vanlandingham et al., 2007). In India, *Cx. tritaeniorhynchus*, *Cx. vishnui*, *Cx. bitaeniorhynchus* and *Cx. univittatus*1, *Cx. pipiens fatigans* and *Aedes albopictus* have been reported as potential vectors of WNV (Ilkal et al., 1997; Varma, 1960).

#### WNV Prevalence in India

In India, seroprevalence of WNV in humans was reported from Mumbai by Banker (1952). WNV neutralizing antibodies have been detected in human sera collected from Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra, Gujarat, Madhya Pradesh, Orissa and Rajasthan (Paramasivan et al., 2003; Risbud et al., 1991; Rodrigues et al., 1980). During 1977, 1978 and 1981 serological evidence of WNV infections have been reported from Vellore and Kolar districts (George et al., 1984). Serological evidence against WNV has been reported among horses in and around Pune City (Paramasivan et al., 2003). In 2010, we screened 1096 equines from 5 states for WNV antibodies by haemagglutination inhibition (HI) and virus neutralization test (VNT). Of these 3.37% were tested positive for WNV antibodies by HI and 3.19% were found positive by VNT, which indicates circulation of WNV among Equine population (Table 1). In southern India, JEV/WNV neutralizing antibodies have been detected in ardeid birds mainly from the pond herons (*Ardeola grayii*) and cattle

egrets (*Bubulcus ibis*), indicating their role in the natural cycle of WNV in India (Rodrigues et al., 1981).

Table 1: Screening of equine serum samples for antibodies to WNV by HI and VNT

Sr. No	State	No. tested	No. Positive by HI (%)	No. Positive by VNT (%)
1.	Punjab	82	2 (2.43)	2 (2.43)
2.	Uttarakhand	312	13 (4.16)	12 (3.85)
3.	Rajasthan	323	12 (3.71)	11 (3.40)
4.	Gujarat	308	4 (1.29)	4 (1.29)
5.	Madhya Pradesh	71	6 (8.45)	6 (8.45)
Total		1096	37 (3.37)	35 (3.19)

WNV strain P-4230 has been isolated from a laboratory worker who got infected while handling the Indian mosquito strain G-2266 and the Egyptian human strain E-101 (Paramasivan et al., 2003). In 2006, WNV belonging to lineage 5 has been detected among humans in the region of Assam to which JEV is endemic (Chowdhury et al., 2014; Khan et al., 2011). An outbreak of acute encephalitis syndrome (AES) due to WNV was reported in Kerala in 2011. The Kerala isolate has the highest nucleotide identity (96.2%) with the Russian isolate (GenBank Acc. no. DQ411030) and belong to Lineage 1 (clade 1a) (Balakrishnan et al., 2013). WNV has been isolated from human beings, frugivorous bat, domestic pigs and mosquitoes in India (Table 2) (George et al., 1984; Paramasivan et al., 2003; Paul et al., 1970; Rodrigues et al., 1980). However, WNV has not been isolated from horses in India.

Isolate No.	Source of Isolation	Location	Year	Lineage	Accession number
WNIG2267Cv	<i>Culex vishnui</i>	Sathuperi	1955	5	AY944243
WNIG16919Cv	<i>Culex vishnui</i>	Kammavanpet	1957	5	AY944245
WNIG22886Cv	<i>Culex vishnui</i>	Sathuperi	1958	5	AY944241
WNI672698H	Human serum	Kasoudi	1967	1a	AY944238
WNI68856B	<i>R. leschenaulti</i> (fruit bat)	Horabail	1968	1a	AY944239
IND804994H	Human	Bangalore	1980	5	DQ256376
WNI80829Cw	<i>Culex whitmorei</i>	Kodikannoor	1980	5	AY639640
WNI80755Cb	<i>Culex bitaeniorhynchus</i>	Kodikannoor	1980	5	AY639641
WNI804987Cv	<i>Culex vishnui</i>	Bangalore	1980	5	AY639642
WNI80245Ct	<i>Culex tritaeniorhynchus</i>	Muduuvadi	1980		AY639639
WNI804994H	Human	Bangalore	1980	5	AY639643
WN80776AnS	<i>Anopheles subpictus</i>	Kodikannoor	1980	5	AY944244
WN80235Cv	<i>Culex vishnui</i>	Arahalli	1980	5	AY944246
WN80897Cw	<i>Culex whitmorei</i>	Arahalli	1980	5	AY944242
WN43478Cv	<i>Culex vishnui</i>	Gownpalli	1981	5	AY944240
WN821622H	Human	Kolar	1982	5	AY944247
WNIRGC07	Human	Assam	2006	5	HQ246154
WNIRTC08	Human	Assam	2006	5	JQ037832
1048813	Human	Kerala	2011	1a	KC601756

Table 2: WNV isolates in India

WNV infection has been reported in pig population around Chandigarh city (Ratho, et al., 1999) and in wild migratory and resident water birds in Eastern and Northern India

(Mishra et al., 2012). WNV has been isolated from various species of mosquito like *Cx. tritaeniorhynchus*, *Cx. vishnui*, *Cx. Bitaeniorhynchus*, etc (Table 1)

### Diagnosis

Diagnosis of WNV infection is based largely on clinical signs, serological methods, nucleic acid based assays and virus isolation/antigen detection.

### Serological Methods

Serological testing remains the primary method of diagnosing WNV infection, mainly based on anti-E antibody detection. However, cross-reactivity of the antibody response with related flaviviruses limits the specificity of serological tests. Plaque reduction neutralization test (PRNT) is the gold standard serological test for WNV detection (Dauphin and Zientara, 2007). It is used as a confirmatory method for detection of WNV-specific neutralizing antibodies from serum or cerebrospinal fluid (CSF). However, PRNT is a laborious test and must be carried out in a biosafety level 3 (BSL-3) facilities. HI test is used for the sero-prevalence of WNV antibodies in population. Demonstration of four-fold rise or drop of antibody titer in paired serum samples confirms the infection. But HI cannot differentiate between closely related flaviviruses and cannot be used as a confirmatory

diagnostic method. Various enzyme-linked immunosorbent assays (ELISA) has been developed for WNV (Blitvich et al., 2003; Choi et al., 2007; Johnson et al., 2003; Kitai et al., 2007; Long et al., 2006). These tests have the advantage of being rapid, reproducible and less expensive. The IgM-antigen capture ELISA (MAC-ELISA) is optimal for the detection of early IgM antibodies, which allows an early diagnosis and the distinction between old and recent infections or vaccinations (De Filette et al., 2012). The MAC-ELISA enables the detection of acute infections since it detects early IgM antibodies in serum or CSF. The IgM antibodies can be detected within 4 to 7 days after the initial exposure and may persist for more than one year (Roehrig et al., 2003; Rossi et al., 2010). National institute of virology, Pune (India) developed MAC-ELISA kit for the diagnosis of WNV, Japanese Encephalitis (JE) and Dengue (DEN) viruses, which is commercialized by Zydus Cadila. We developed a panel of murine monoclonal antibodies (MAbs) against WNV and using these mAbs, developed a blocking-ELISA for epitope detection of WNV in Horses (Gupta et al., 2013).

Vaccine	Antigen	Stage of Development
ChimeriVax (Sanofi)	Yellow fever PrM-E substituted by WNV prM-E	Phase II
Chimeric WN/DEN4	Chimeric DV4 backbone expressing WNV PrM/E	Phase I
WN-80E	Soluble E lacking the trans membrane domain	Phase I
STF2Δ.EIII	S. typhimurium flagellin fused to E domain III	Evaluated in mice
rWNV-ET	Truncated protein E	Evaluated in mice and horses
SRIP	prM-E VLPs	Evaluated in mice and horses
CAdVax-WNVII	C, prM, E and NS1 expressed in adenovirus	Evaluated in mice
FLU-NA-DIII	E domain III inserted into NA of influenza	Evaluated in mice

Table 3: West Nile virus candidate vaccines

### Nucleic Acid Based Assays

WNV can be detected by conventional reverse-transcription polymerase chain reaction (RT-PCR), real-time PCR and multiplex reverse transcription-PCR ligase detection reaction (Johnson et al., 2001; Linke et al., 2007; Rondini et al., 2008; Shi and Kramer, 2003). A SYBR Green based assay that could detect 100% of the different WNV target region variants have been developed (Papin et al., 2004). But this SYBR based assay has lower sensitivity as compared to WNV-specific TaqMan RT-PCR assays (Johnson et al., 2010). The two most popular alternatives to SYBR Green are TaqMan and molecular beacons, both of which use hybridization probes and rely on fluorescence resonance energy transfer (FRET) for quantification (Jimenez-Clavero et al., 2006; Lanciotti et al., 2000). Parida and co-workers (2004) developed a loop-mediated isothermal amplification (LAMP) assay for WNV, which is relatively inexpensive. In India during 2010-11, conventional RT-PCR, real-time RT-PCR and RT-LAMP assays were used to detect WNV associated with multifocal retinitis in patients from southern India (Shukla et al., 2012).

### Antigen Detection/Virus Isolation

It is the method of choice for detection of WNV in vertebrate/ mosquito pools/avian samples. Virus can be isolated from CSF, blood or brain tissues in cell cultures viz: Vero/BHK 21/RK-13/mosquito (AP61) cell lines (Castillo-Olivares and Wood, 2004). Antigen capture ELISA has been developed for the detection of WNV using NS-1 (Chung

and Diamond, 2008; Macdonald et al., 2005). A membrane-based electrochemical nano-biosensor has been developed for the detection of WNV that recognizes viral particles or virus E protein during the early stage of infection (Nguyen et al., 2009). In India, WNV has been isolated by intra-cerebral inoculation in 3-day old infant mice; inoculation in BHK-21 and Vero cell lines (Balakrishnan et al., 2013; Bondre et al., 2007; Chowdhury et al., 2014).

### Vaccination

Vaccination is the primary method of reducing the risk of infection from WNV. At present, there are no commercially available vaccines for human use. A number of vaccine strategies have been explored and some human candidate vaccines have been evaluated in Phase I and II clinical trials (Table 3) (Iyer and Kousoulas, 2013).

Compared to WNV vaccine for human, there are effective licensed vaccines for horses (De Filette et al., 2012). Fort Dodge Animal Health (subsidiary of Pfizer) developed a vaccine under the trade name West Nile-Innovator® which is formalin-inactivated whole West Nile virus (Ng et al., 2003). This vaccine is currently commercialized in the USA and is quite effective. Another killed virus vaccine (Vetera® WNV vaccine) developed by Boehringer Ingelheim Vetmedica is also licensed by the United States Department of Agriculture (USDA). A third commercialized WNV vaccine in the United States for horses is Recombitek® Equine West Nile Virus Vaccine (Merial, now Sanofi Aventis), which is a chimeric recombinant canarypoxvirus

vaccine (El Garch et al., 2008). In 2005, Fort Dodge Animal Health/Pfizer developed a DNA based WNV vaccine under the trade name of West Nile–Innovator® DNA. The vaccine contains an unformulated plasmid DNA encoding the prM and E protein of WNV and MetaStim™ as adjuvant. This vaccine has recently been discontinued by Pfizer (Brandler and Tangy, 2013).

### Concluding Remarks

WNV infection is emerging as an important infection of human and animals across the globe. In India, WNV infection has been reported from humans, pigs, bats and birds. A few studies indicate the seroprevalence of WNV among horses in India. Mosquitoes of mainly *Culex* species and sometime *Anopheles subpictus* are the main vectors of WNV in India. Some data has emerged during last 5 decades on epidemiology of WNV in India. However, there is a need for systematic study to know the status of WNV infection in human and different animals, particularly horses in India. Role of mosquitoes and various birds in spread of WNV in newer geographical regions of India also needs further investigations.

Research has gained pace regarding the public health significance of the WNV infection. However, there is greater amount of serological cross-reactivity of WNV with related flaviviruses circulating in a region. For instance, in India WNV and Japanese encephalitis virus (JEV) both are endemic and co-circulate in human and animal population. Therefore, there is need for development of sensitive and rapid sero-diagnostics, which can clearly discriminate WNV from JEV in India. Development of sensitive and rapid molecular diagnostics should be encouraged for early detection of infection. The environmental factors play critical role in emergence, maintenance and transmission of the WNV to newer geographical regions. Hence, impact of changing environment in the ecology of WNV needs to be investigated. There is no specific treatment for WNV; therefore strong surveillance system needs to be developed for understanding the pattern of disease distribution. Efforts for development of immuno-prophylactics for human and animals should be done for control of WNV infection. It is hoped that the increase in knowledge of interaction of virus, vector and host will provide new insights for the therapeutic and preventive measures.

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### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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