Letter

A Brief View on Molecular Diagnosis and Surveillance of West Nile Virus

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Introduction

West Nile Virus (WNV) is an important zoonotic agent having a wide host range. Due to its emergence with increased virulence in a wide geographical range, its monitoring becomes imperative. Development of more rapid and sensitive molecular techniques for instance Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR), reverse transcription loop-mediated isothermal amplification (RT-LAMP) and Nucleic Acid Sequence-Based Amplification (NASBA) assays are vital for detection of the virus. Various surveillance techniques according to epidemiological, climatic and geographical conditions in the exposed area have also been developed. The surveillance can be set up at different levels of the WNV transmission cycle using birds, horses and mosquitoes as sentinels.

West Nile Virus (WNV) is one of the imperative emerging infectious agents with zoonotic potential. It affects wide varieties of hosts, produces ranges of clinical manifestations and has emerged with increased severity with different features and patterns of virulence. WVN is a positive sense single-stranded RNA enveloped virus of the genus *Flavivirus*, family *Flaviviridae*.

Phylogenetic studies have identified two main lineages of WNV. Strains from Lineage 1 are present in Africa, India, Australia and the Western Hemisphere and have been responsible for recent epidemics in Europe, the Mediterranean basin, the Americas; and strains from Lineage 2 have been reported only in sub-Saharan Africa and have not been associated with epidemic transmission [(1)].

The natural cycle of all members of the JE antigenic complex of *Flaviviruses* involves birds as the main amplifying host and several species of mosquitoes as the vectors, ornithophilic mosquitoes, particularly, *Culex* species. WNV has been detected in at least 61 species of North American mosquitoes and 328 avian species in United States [(2)].

WNV infections acquired through consumption of infected tissues have been reported for birds, mammals and reptiles. Humans and other mammals serve as dead-end hosts and do not sufficiently amplify virus for mosquito transmission, although they may transmit or acquire virus in utero, through breast milk, via blood transfusion or organ transplantation, or through occupational exposure [(3, 4)].

Diagnosis

The definitive detection method for WNV in vertebrate, mosquito pools and avian samples remain viral isolation which can be performed from Cerebrospinal Fluid (CSF), blood or tissues in infected cell cultures. Infected cell culture supernatants or preparations from WNV infected Suckling Mouse Brains (SMB) are antigens classically used for WNV serodiagnosis. Alternatively, recombinant antigens such as the envelope glycoprotein E, Virus Like Particles (VLP), or the non-structural NS1, NS3 and NS5 proteins may be used in different assay formats in the absence of particular containment facilities [(5)].

Antibody testing in human or animal sera is of large usefulness for diagnosing WNV infection but cross-reactivity limits diagnostic specificity. WNV specific IgM and IgG capture ELISA tests and the Plaque Reduction Neutralization Test (PRNT) detecting WNV specific neutralizing antibodies in CSF and serum remains the assay required for confirmation of *flavivirus* infections. MAC-ELISA test is the most efficient for detection of IgM antibody and is valuable for serosurveillance. The new microsphere immunoassay provides a sensitive and rapid alternative to traditional ELISA that detects antibodies to flavivirus E proteins [(6)].

The presence of the virus can be confirmed by nucleic acid detection. A sensitive and WNV-specific reverse transcription and nested PCR method has been used successfully [(7)]. In addition to greatly enhancing detection sensitivity, the shorter turn-around time of real-time PCR has made it a more favoured diagnostic technique. A

sensitive real-time PCR assay, incorporating Fluorescence Resonance Energy Transfer (FRET) probes have been designed for the rapid and simultaneous detection and genotyping of WNV [8].

Immunohistochemistry on CNS tissues using WN-specific MAb and Antigen capture ELISA tests to confirm the presence of WNV in avian tissues and mosquito pools are very imperative diagnostic methods. Advanced nucleic acid based techniques like TaqMan reverse transcriptase-PCR assay and Nucleic Acid Sequence Based Amplification (NASBA) assays have demonstrated a greater sensitivity than the traditional RT-PCR method [9]. A one step, single tube Real-time reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay is another novel method of gene amplification developed for rapid detection of the envelope gene of WNV [10].

Surveillance

The surveillance of WNV can be performed by using passive surveillance system or by active surveillance depending on epidemiological, climatic and geographical conditions in the exposed area. Most of the threatened countries have organized a passive surveillance with horses, birds and humans.

Horses serve as good sentinels for WNV infection surveillance because they are easily identifiable; their role in the epidemiological cycle of WNV; being dead-end hosts like humans; low cost easy to maintain facility to capture and sample horses; and the availability of serological tools are to do surveillance.

Birds have always been considered useful candidates as sentinels for the presence of this virus in a geographical area. Mosquito testing is not a practical method for routine surveillance of transmission; because the proportion of WNV positive mosquito pools in wild populations is very low even when transmission rates are high.

In conclusion, emergence of WNV as a major cause of public health concern and development of advance molecular techniques for the diagnosis and surveillance has enhanced our understanding of the pattern of development and spread of WNV in different geographical regions of the world.

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